User's Guide

NEO-SENSITABS™

SUSCEPTIBILITY TESTING

19th Ed.
2007/2008

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USER's GUIDE NEO-SENSITABS™

SUSCEPTIBILITY TESTING 19th Ed. 2007/2008

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PREFACE

The 19th Ed. 2007/2008 of the NEO-SENSITABS™ User's Guide contains updated text, tables and references, all informative information when using Neo-Sensitabs tablets for susceptibility testing. A table giving the changes from the 19th Ed. 2007/2008 is available at our website.

The different interpretation tables following the CLSI (formerly NCCLS) recommendations have been updated according to the latest information of the CLSI described in "Performance Standards for Antimicrobial Susceptibility Testing", 15th Informational Suppl., **M100-S17**, 2007.

Furthermore, the User's Guide includes updated Zone Diameter Interpretative Standards according to CRG, SRGA, AFA, Denmark, SFM, DIN and BSAC.

Information and recommendations given by susceptibility testing standardization groups or organizations in different European countries provide supplementary knowledge of specific considerations and decisions made in each country.

In the future, the use of common European MIC breakpoints would permit the different countries (and laboratories) to use their separate techniques (including inoculum, media, tablets or disks, automatic methods etc.) and obtain comparable results, because the techniques used would be standardized according to common European MIC breakpoints (1). The European Committee on Antimicrobial Susceptibility Testing (EUCAST) has been reorganized in 2001/2002 giving national breakpoint committees (at present France, Germany, Norway, Sweden, the Netherlands and the UK) a greater role. One of the major tasks of the EUCAST is to harmonize MIC breakpoints across Europe and the process is in progress (2). At the moment MIC-breakpoints for aminoglycosides, cephalosporins, carbapenems, monobactams, fluoroquinolones, glycopeptides, oxazolidinones, tigecycline, and daptomycin have been harmonised though EUCAST. If included in national recommendations, they are included in the interpretation tables I-VIII (3).

The User's Guide is available at our website www.rosco.dk and updated information is continuously included.

Rosco Diagnostica A/S is welcoming any feedback and questions on susceptibility testing from users directly (info@rosco.dk) or through our representatives.

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August 2007

References:

1) Kahlmeter G.: Breakpoints in Europe: a future perspective. 42nd ICAAC. Presentation 1022, 2002.
2) Kahlmeter G. et al: European harmonization of MIC breakpoints for antimicrobial susceptibility testing of bacteria. J. Antimicr. Chemother. **52**, 145-148, 2003.
3) http://www.srga.org/eucastwt/MICTAB/index.html

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1 Susceptibility Testing in General

Susceptibility testing is of great importance to the hospital physician and the practitioner in the treatment of infectious diseases. Knowledge of susceptibility to different antibiotics of an infectious agent, facilitates the choice of the most effective antibacterial agents.

In most clinical laboratories an agar diffusion (disk/tablet) method is used routinely for testing the susceptibility of common rapidly growing bacterial pathogens. It is generally accepted that reliable results can be obtained by the diffusion test, when a standardized methodology is used, and the diameter of the inhibition zones has been correlated with minimal inhibitory concentrations (MIC). The interpretation of zone diameters by the diffusion method, should be based on regression lines. A regression line, for a certain antibiotic contained in a disk/tablet, is a graphic expression of the correlation existing between MIC's and the diameters of inhibition zones obtained with a number of bacterial strains. These lines represent a control of the standardization of the method, showing that the interpretative zone diameters correspond to MIC values in agreement with internationally accepted standards. The availability of regression lines makes it possible for the microbiologist to convert the results obtained by the diffusion method (susceptible, intermediate, resistant) into more quantitative results, i.e. approximated MIC values.

An accurate diffusion method requires:
- A well-standardized technique including control strains (e.g. *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853 and *Enterococcus faecalis* ATCC 29212) enabling control of inoculum and media variations.
- Well-traced regression lines, which can be controlled, because there is a correlation between the slope of the regression line and the molecular weight of the antimicrobial tested.
- Separate regression lines for rapidly growing bacteria and slow-growing species.

Regression lines for Neo-Sensitabs have been prepared using the agar dilution method to determine the MIC values. For the aminoglycosides (amikacin, gentamicin, netilmicin, tobramycin) and polymyxins (colistin) we have also determined MIC's by the broth dilution method in order to correct for the amount of antimicrobial bound to the agar. The above mentioned antimicrobials are strongly bound to the sulphate groups of the agar, and consequently, different MIC values as well as zone sizes may be obtained depending on the purity of the agar used. It is, therefore, important to control the quality of the agar used in the diffusion test, and this may be achieved by controlling zone sizes with control ATCC strains.

Separate interpretation tables have been prepared for slow-growing and bacteria with special requirements (Haemophilus, Streptococci, Moraxella, Neisseria, Pneumococci, and Anaerobes).

This booklet describes methods, quality control and interpretative criteria recommended in different countries for diffusion susceptibility tests with Neo-Sensitabs. When new problems are recognized or improvements are developed, changes will be incorporated in future editions of the booklet and also distributed as informational supplements.

References:
1) Casals J.B, Gylling Pedersen O.: Tablet sensitivity testing: a comparison of different methods. Acta Path. Microbiol. Scand, sect B, **80B**, 806-816, 1972.
2) Schumacher H. et al: A procedure for evaluation and documentation of susceptibility test methods using the susceptibility of Klebsiella pneumonae to ciprofloxacin as model. J. Antimicr. Chemother., **48**, 493-500, 2001.

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2 Characteristics of NEO-SENSITABS

Neo-Sensitabs are produced according to the guidelines of WHO (1). The tablets are 9 mm in diameter and each Neo-Sensitabs is printcoded for safe identification. The tablets are manufactured with the aid of microbially inert auxiliary substances by a dry process using crystalline antimicrobials. This procedure results in very uniform tablets, homogenous in their content of active ingredients, and with an extraordinary stability (usually not less than 4 years shelf-life at room temperature (2)).
Neo-Sensitabs are standardized according to the MIC-breakpoints recommended by "Susceptibility Testing Standardization Groups" in several countries (e.g. Holland, Norway, Sweden, France, Germany, UK and Denmark) including the CLSI (3).

All antimicrobials have received new letter codes (see chapter 3), because in order to achieve optimal recognition and zone measurements with automatic instruments a 5-digit code for each Neo-Sensitabs type has been chosen.

References:
1) World Health Organization: Expert Committee on Biological Standardization. Requirements for antibiotic susceptibility tests. Requirements for Biological Substances No. **26**, Geneva, September 1981.
2) Gylling Pedersen O.: Standardizing, manufacture, and control of Neo-Sensitabs. Acta Clin. Belg., **28**, 139-149, 1973.
3) NCCLS: Performance Standards for Antimicrobial Disk Susceptibility Testing. 8th Ed. **M2-A8** January 2003.
4) Stes P. et al: Cefepime activity against Ps. aeruginosa: evaluation of Etest and two disc diffusion methods. J. Antimicro. Chemother., **38**, 707-711, 1996.
5) Engberg J. et al: Comparison of two agar dilution methods and three agar diffusion methods, including the Etest, for antibiotic susceptibility testing of thermophilic Campylobacter species. Clin. Microbiol. and Infect., **5**, 580-584, 1999.
6) Koeth L. et al: Quality control study evaluating the performance of daptomycin and daptomycin/calcium disks and Etest. Clin. Microbiol. Infect., **10**, Supp. 3, 541, 2004.
7) Denis O. et al: Polyclonal emergence and importation of CA-MRSA strains harbouring Panton-Valentine leucocidin genes in Belgium. JAC, **56**, 1103-06, 2005.

2.1 Storage of NEO-SENSITABS

Neo-Sensitabs are stored at room temperature (both unopened and cartridges in use).
Very few exceptions (Cefpodoxime, Cefepime, Cefepime+Clavulanate, Temocillin, Ticarcillin, Ticarcillin+Clavulanate, Amphotericin B, Caspofungin) must be stored in the refrigerator. With these Neo-Sensitabs (labelled "STORE AT 2-8°C") the following precautions must be taken:

1) Unopened cartridges (stock): Store in refrigerator, 2-8 °C. (Expiry date on the label and cartridge).

2) Cartridges that have been opened: Store at room temperature for up to 2 months.

3) Cartridges placed in dispenser: Store in dispenser at room temperature for up to 2 months.

If an opened cartridge (labelled 2-8°C) is not used up within 2 months, it should be kept in the refrigerator. Each time the cartridge is taken from the refrigerator, it must be allowed time to reach room temperature (30-60 min.) before it is opened, in order to avoid water condensation on the tablets. Cartridges (labelled 2-8°C) that have been opened and are kept in the refrigerator should be used within 6 months.
With Neo-Sensitabs stored at room temperature (labelled "store below 25 °C), cartridges that have been opened (or are placed in a dispenser), should be stored at room temperature and used within 12 months of opening date.
The stability of antimicrobials in paper disks is decreased compared to Neo-Sensitabs. The CLSI (1) recommends frozen storage of paper disks containing beta-lactam antibiotics. In case of Imipenem, Cefaclor and Clavulanic acid combinations, paper disks should be stored frozen until the day of use.

In a comparative stability study between Neo-Sensitabs and Oxoid paper disks (2), it was observed that disks containing Ticarcillin 75 µg , lost activity after 15 days at 4-6 °C, while Ampicillin 10 µg and Amoxycillin + Clavulanate 20+10 µg disks lost activity after one month at 4-6 °C.The corresponding Neo-Sensitabs were stable at room temperature (and at 4-6 °C) during the study period of six months.

Steward et al (3) noticed overdetection of imipenem/meropenem resistance in the project ICARE, most probably due to inactivation of the reagents used (Vitek, disk diffusion etc.) and recommended the use of a second independent antimicrobial susceptibility testing method to validate carbapenem-intermediate and resistant strains.

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References:

1) NCCLS: Performance Standards for Antimicrobial Disk Susceptibility Testing, 8th Ed. **M2-A8**, January 2003.
2) del Cuerpo M. et al: Stability of beta-lactam antibiotics in paper disks and tablets used in the diffusion test. Rev. Esp. Quimioter., **10**, nr. 3,1997 (Spanish).
3) Steward C.D. et al: Antimicrobial susceptibility testing of carbepenems: multicenter validity testing and accuracy levels of 5 antimicrobial test methods for detecting resistance in Enterobacteriaceae and Pseudomonas aeruginosa isolates. J. Clin. Microbiol., **41**, 351-358, 2003.

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3 NEO-SENSITABS Range

3.1 Antibacterials

Neo-Sensitabs	Identification code Neo-Sensitabs	Diffusible amount of Antimicrobial
A. Penicillins (Penams)		
PENICILLIN LOW	PEN.L	5 µg
AMPICILLIN 33 µg	AMP33	33 µg
AMPICILLIN 2.5 µg	AMP.L	2.5 µg
AMOXYCILLIN	AMOXY	30 µg
METHICILLIN	METHI	29 µg
OXACILLIN 1 µg	OXA.1	1 µg
OXACILLIN 5 µg	OXA.5	5 µg
CLOXACILLIN 500 µg (AmpC test) (Diatabs)	CL500	500 µg
MECILLINAM (Amdinocillin)	MECIL	33 µg
TICARCILLIN	TICAR	75 µg
PIPERACILLIN	PIPER	100 µg
TEMOCILLIN	TEMOC	30 µg
B. Beta-lactam/ Beta-lactamase Inhibitor Combinations		
AMOXYCILLIN+CLAVULANATE (Augmentin)	AM+CL	30+15 µg
AMPICILLIN+SULBACTAM	AM+SU	30+30 µg
TICARCILLIN+CLAVULANATE (Timentin)	TI+CL	75+15 µg
PIPERACILLIN+TAZOBACTAM	PI+TZ	100+10 µg
CEFTAZIDIME+CLAVULANATE	CZ+CL	30+10 µg
CEFEPIME+CLAVULANATE	CP+CL	30+10 µg
IMIPENEM+EDTA	IM+ED	15+750 µg
BORONIC ACID (Diatabs)	BORON	250 µg
CEFTAZIDIME+DIPICOLINIC ACID	CAZ+D	30+250 µg
DIPICOLINIC ACID (Diatabs)	D.P.A.	250 µg
C. (1) Cephalosporins (Cephems)		
CEPHALOTHIN	CLOTN	66 µg
CEFACLOR	CCLOR	30 µg
CEFADROXIL	CFDRO	30 µg
CEFAZOLIN	CFZOL	60 µg
CEPHALEXIN	CFLEX	30 µg
CEPHRADINE	CFRAD	60 µg
CEFUROXIME	CEFUR	60 µg
CEFONICID	CFCID	30 µg
CEFIXIME	CFFIX	30 µg
CEFPODOXIME	CFPOX	30 µg
CEFOTAXIME	CFTAX	30 µg
CEFTAZIDIME	CEZDI	30 µg
CEFTRIAXONE	CETRX	30 µg
CEFTIZOXIME	CEZOX	30 µg
CEFTIOFUR (Vet.)	CFTIO	30 µg
CEFEPIME	CFEPM	30 µg

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Neo-Sensitabs	Identification code Neo-Sensitabs	Diffusible amount of Antimicrobial
CEFPIROME	CFPIR	30 µg
CEFSULODIN	CFSUL	30 µg
CEFQUINOME (Vet.)	CFQUI	30 µg
CEFTOBIPROLE (BAL 9141)	CFBIP	30 µg
C. (2) Cephamycins and Oxacephems		
CEFOXITIN	CFOXT	60 µg
C. (3) Cephalosporins active against MRSA		
CEFTOBIPROLE (investigational drug)	CFBIP	30 µg
CEFTAROLINE (investigational drug)	CFARL	30 µg
D. Penems and Carbapenems		
IMIPENEM	IMIPM	15 µg
MEROPENEM	MEROP	10 µg
ERTAPENEM	ERTAP	10 µg
DORIPENEM (investigational drug)	DORIP	10 µg
FAROPENEM (investigational drug)	-----	-----
E. Monobactams		
AZTREONAM	AZTRM	30 µg
F. Aminoglycosides		
STREPTOMYCINS 100 µg	ST100	100 µg
STREPTOMYCIN 500 µg (HLR)	ST500	500 µg
KANAMYCIN 100 µg	KANAM	100 µg
KANAMYCIN 500 µg (HLR)	KA500	500 µg
NEOMYCIN (Framycetin)	NEOMY	120 µg
AMIKACIN	AMIKA	40 µg
GENTAMICIN 40 µg	GEN40	40 µg
GENTAMICIN 250 µg (HLR)	GN250	250 µg
NETILMICIN	NETIL	40 µg
TOBRAMYCIN	TOBRA	40 µg
SPECTINOMYCIN	SPECT	200 µg
APRAMYCIN (Vet.)	APRAM	40 µg
ISEPAMICIN 30 µg	ISP30	30 µg
G. Tetracyclines		
TETRACYCLINES 80 µg (Oxytetracycline)	TET80	80 µg
TETRACYCLINES 10 µg	TET10	10 µg
DOXYCYCLINE	DOXYC	80 µg
MINOCYCLINE	MINOC	80 µg
TIGECYCLINE (investigational drug)	TIG15	15 µg
H. Chloramphenicol and derivatives		
CHLORAMPHENICOL 60 µg	CLR60	60 µg
CHLORAMPHENICOL 10 µg	CLR10	10 µg
FLORFENICOL (Vet.)	FFC30	30 µg
I. Macrolides, Lincosamides, Streptogramines, Ketolides and Oxazolidinones		
ERYTHROMYCIN	ERYTR	78 µg
AZITHROMYCIN	AZITR	30 µg
CLARITHROMYCIN	CLARI	30 µg
LINCOMYCIN	LINCO	19 µg

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Neo-Sensitabs	Identification code Neo-Sensitabs	Diffusible amount of Antimicrobial
CLINDAMYCIN 25 µg	CLIND	25 µg
SPIRAMYCIN	SPIRA	200 µg
PRISTINAMYCIN	PRIST	30 µg
VIRGINIAMYCIN	VIRGI	30 µg
QUINUPRISTIN/DALFOPRISTIN	SYN15	15 µg
TELITHROMYCIN	TEL15	15 µg
LINEZOLID	LINEZ	30 µg
LINCO-SPECTIN (Vet.)	LI+SP	15+200 µg
TYLOSIN (Vet.)	TYLOS	150 µg
TILMICOSIN (Vet.)	TILMI	80 µg
PIRLIMYCIN (Vet.)	PIRLI	10 µg
J. (1) Glycopeptides		
VANCOMYCIN 5 µg	VAN.5	5 µg
J. (2) Lipoglycopeptides		
TEICOPLANIN	TPN30	30 µg
DALBAVANCIN (investigational drug)	DALBA	30 µg
TELAVANCIN (investigational drug)	TELAV	30 µg
J. (3) Cyclic lipopeptides		
a) Gram positive spectrum:		
DAPTOMYCIN (+ Ca)	DAPCa	30 µg
BACITRACIN	BACIT	40 units
b) Gram negative spectrum:		
COLISTIN 10 µg	CO.10	10 µg
POLYMYXINS 150 µg (Colistin)	CO150	150 µg
K. Sulphonamides and similars		
SULPHONAMIDES	SULFA	240 µg
TRIMETHOPRIM	TRIME	5.2 µg
TRIMETHOPRIM+SULFA	TR+SU	5.2+240 µg
L. Nitrofurans		
NITROFURANTOIN	NITRO	260 µg
FURAZOLIDONE	FURAZ	50 µg
M. Quinolone derivatives		
NALIDIXAN	NALID	130 µg
CINOXACIN	CINOX	30 µg
FLUMEQUINE (Vet.)	FLUME	30 µg
OXOLINIC ACID	OXOLI	10 µg
PIPEMIDIC ACID	PIPEM	30 µg
CIPROFLOXACIN 10 µg	CIP10	10 µg
CIPROFLOXACIN 0.5 µg	CIP.L	0.5 µg
MOXIFLOXACIN	MOXIF	5 µg
GATIFLOXACIN	GATIF	5 µg
ENROFLOXACIN (Vet.)	ENROF	10 µg
LEVOFLOXACIN	LEVOF	5 µg
MARBOFLOXACIN (Vet.)	MARBO	5 µg
NORFLOXACIN	NORFX	10 µg
OFLOXACIN	OFLOX	10 µg
PEFLOXACIN	PEFLX	10 µg

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Neo-Sensitabs	Identification code Neo-Sensitabs	Diffusible amount of Antimicrobial
N. Others		
FOSFOMYCIN (+G6-P)	FOSFO	70+40 µg
FUCIDIN	FUCID	100 µg
METRONIDAZOLE 16 µg	MTR16	16 µg
MUPIROCIN	MUPIR	10 µg
NOVOBIOCIN 5 µg	NOV05	5 µg
RIFAMPICIN	RIFAM	30 µg
TIAMULIN (Vet.)	TIAMU	30 µg
PENICILLIN/NOVO (Vet.)	PEN+N	10 U/30 µg

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3.2 Antifungals

Neo-Sensitabs	Identification code Neo-Sensitabs	Diffusible amount of Antimicrobial
AMPHOTERICIN B	AMPHO	10 µg
CICLOPIROX	CICLO	50 µg
CLOTRIMAZOLE	CLOTR	10 µg
ECONAZOLE	ECONZ	10 µg
FLUCONAZOLE	FLUCZ	25 µg
5-FLUOROCYTOSINE 10 µg	FLU10	10 µg
5-FLUOROCYTOSINE 1 µg	FLU.1	1 µg
GRISEOFULVIN	GRISE	25 µg
ISOCONAZOLE	ISOCN	10 µg
ITRACONAZOLE	ITRAC	8 µg
KETOCONAZOLE	KETOC	15 µg
MICONAZOLE	MICON	10 µg
NATAMYCIN	NATAM	50 µg
NYSTATIN	NYSTA	50 µg
TERBINAFINE	TERBI	30 µg
VORICONAZOLE	VOR.1	1 µg
CASPOFUNGIN (investigational drug)	CASP5	5 µg
POSACONAZOLE (investigational drug)	POSAC	5 µg

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4 Susceptibility Test Media

In order to achieve satisfactory results, a proper medium for susceptibility testing must be used. It should fulfil at least two requirements:
- The composition of the medium should give sufficiently good growth conditions for the strains to be tested.
- The medium must not contain material interfering with the test itself or with any antimicrobial contained in Neo-Sensitabs.

The ideal medium for susceptibility testing has not been found yet, and this is one of the reasons why different media are used in different countries. Mueller-Hinton agar is recommended by the FDA and the CLSI in the U.S. (1) and is routinely used in several European countries.

Variations in Mueller-Hinton agar from different manufacturers were first recognized with tetracyclines and amino-glycosides. Variation in Mg and Ca, will affect results of aminoglycoside and tetracycline tests with *Ps. aeruginosa*. Excess zinc ions may reduce zone sizes of carbapenems. Excessive cation content will reduce zone sizes, whereas low cation content may result in unacceptable large zones of inhibition (1). Ca and Mg should be available in the medium, in the form of soluble salts.

Later differences were found in thymidine content affecting testing of trimethoprim and methicillin-resistant staphylococci (2,3).

Most agar media contain small amounts of sulphonamide and trimethoprim antagonists that may affect the results of susceptibility testing (especially if blood is not added) when low content disks are used, while Neo-Sensitabs are less affected (4). Susceptibility test media should contain less than 0.03 mg/l thymidine, otherwise small colonies are seen inside the trimethoprim zone. If the medium contains slightly more thymidine than recommended, it is possible to reduce the concentration by adding thymidinephosphorylase: 0.025 to 0.1 IU enzyme/ml medium or 5% haemolyzed horse blood, which contains the same enzyme.

Reducing the thymidine content of Mueller-Hinton agar for clearer sulphonamide and trimethoprim zones might have an inhibitory effect on the growth of some staphylococci. This phenomenon may also be related to problems in detecting resistance to oxacillin/methicillin in staphylococci. Consequently, to make sure that a certain Mueller-Hinton agar is useful for susceptibility testing, it must fulfil the following criteria:
- It shows acceptable batch to batch reproducibility for susceptibility testing.
- Zone diameters with the 4 control strains must fall within the specified limits.
- Ability to detect methicillin-resistance in staphylococci. (Quality control with *S. aureus* ATCC 43300).
- Ability to produce clear inhibition zones within the control limits, around Trimethoprim and Trimethoprim+Sulfa with enterococci. (Quality control with *E. faecalis* ATCC 29212).

Only Mueller-Hinton medium formulations, that have been tested according to, and that meet, the acceptance limits described in the CLSI document M6, should be used (1).

Neo-Sensitabs have been standardized with the following susceptibility test media: Mueller-Hinton Agar, Iso-sensitest Agar, and Danish Blood Agar.

The pH value of each batch of agar medium should be checked when the medium is prepared. The agar medium should have a pH of 7.2 - 7.4 at room temperature after gelling.

If the pH is too low, certain antimicrobials will appear as having lower potency (e.g. aminoglycosides, macrolides) and the strains will appear as more resistant, while other antimicrobials may appear as having excessive activity (e.g. penicilins) and the corresponding strains will appear as more susceptible. If the pH is too high, the opposite effects can be expected (1).

The thickness of the agar must be 4 mm ± 1 mm.

References:
1) NCCLS: Performance Standards for Antimicrobial Disk Susceptibility Tests. **M2-A8**. 8th Ed., January 2003.
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5 Primary vs. Pure Culture Susceptibility Testing Early Reading of the Antibiogramme

Direct (or primary) susceptibility tests, in which tablets or disks are applied to plates inoculated from the specimen, are widely used in Denmark and the U.K., but their reliability is often questioned by microbiologists in other countries. The advantages of these tests are:
a) speed as results may be available on the following day; b) identification of small numbers of resistant organisms in a predominantly susceptible population and c) their value as selective media in mixed cultures (1).

1) Urine

Primary susceptibility tests of **urine specimen** are particularly successful (5).

In urgent clinical situations, physicians may request more rapid information to guide their selection of antimicrobial agents. Therefore, it is common practice in some laboratories to report presumptive susceptibilities after 6-8 hours incubation (9). Midtvedt & Midtvedt (6) found a good correlation between results obtained after 8 hours and 18-24 hours incubation, respectively. In most cases the zones of inhibition were found to be somewhat larger at 18-24 hours than after 8 hours. None of the strains observed to be susceptible after 8 hours incubation was found to be resistant after 18-24 hours.

Saha et al (9) using rapid but low-cost susceptibility testing methods (disk diffusion) obtained antibiogram results (8 hours incubation) identical to those obtained by conventional methods.

2) Blood cultures

Previous studies have shown an agreement of 94.6 % or higher when comparing direct (primary) susceptibility testing by the diffusion method utilizing positive blood cultures as inoculum and standard diffusion susceptibility test methods (2,3,4).

Coyle et al (7) used a combination of direct inoculation from blood cultures and early readings (6 hours) of disk diffusion. 91% of the tests with gram-positive organisms and 86% gram-negatives were in agreement with standard results. Early reading results must be confirmed by re-reading after 18-24 hours incubation or by standard susceptibility testing.

In clinical emergencies when the gram strain suggests that the infection is due to a single species, direct susceptibility tests may be performed and the results reported as preliminary (8). Once isolated colonies become available from the initial culture, a follow-up standardized test should be performed.

Navon-Venecia et al. (11) inoculated 0.2 ml from incubated aerobic bottles (blood culture) into Mueller-Hinton agar plates and tested for ESBL production by the disk method. High concordance between direct testing and the standard protocol was achieved. Weinbren et al (13) reported ESBL results after 5-6 hours' incubation with *E. coli* and *Klebsiella* spp.

3) Faeces

For detecting the faecal carriage of antimicrobial-resistant *E. coli*, a faecal swab was directly plated onto a McConkey plate and antimicrobial disks applied onto the seeded plate. The same swab was tested with a conventional method. The rapid screening method showed a good specificity and good concordance with the conventional method. (Paradisi et al. (12), Kronwall et al. (15)).

Bartolini et al. (16) using a direct plating method of fecal samples on McConkey agar studied the status on antimicrobial resistance in commensal *E. coli* in preschool children from low-resource countries. This inexpensive, sensitive and simple method detected high resistance rates of *E. coli* to ampicillin (95 %), Trim+Sulfa (94 %), Tetracycline (93 %), and Chloramphenicol (70 %). 35 % of the strains were resistant to Nalidixic acid.

4) Sputum

Cercenado et al (10) in patients with ventilator-associated pneumonia evaluated the Gram-strain and direct susceptibility test by the diffusion method (E-test) on respiratory samples. Results of the difusion test correlated with the microdilution method in susceptibility categories in all cases with *S. aureus*, *P. aeruginosa* and *E. coli*.

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For the bacteriological analysis of sputum from cystic fibrosis patiens, colonised by *P. aeruginosa*, sputum is fluidized with acetylcystein and further diluted 1/20 with saline. Inoculate MH-plate + 5 % blood. Add antimicrobial disks (tablets): Ticarcillin, Ceftazidime, Aztreonam, Imipenem, Tobramycin, Ciprofloxacin, Polymyxins (colistin) and C-390. After incubation overnight, look for suspicious colonies inside the inhibition zones. Identify and perform antibiograms (14). *S. maltophilia* will show resistance to imipenem, *P. aeruginosa* resistance to C-390, *A. xylosoxydans* resistance to tobramycin and *B. cepacia* resistance to colistin.

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6 Inoculum Standardization and Prediffusion Method

6.1 Inoculum Standardization: ICS and CLSI (Kirby-Bauer)

The specimen must be fully typical of the site of infection, i.e. every effort should be made to obtain a representative sample of the relevant pathogenic bacteria.

The density of the inoculum is one of the variables that will have a great influence on the size of the zones of inhibition. With primary cultures, the inoculum may vary to a large degree, i.e. from few colonies to a dense growth. Our studies with Neo-Sensitabs have shown that too sparse an inoculum will give too large zones, and too dense an inoculum will give too small zones compared to the interpretation charts.

6.1.1 Inoculum according to ICS

1) When secondary (pure) cultures are used, the inoculum may be standardized as recommended by the ICS (1) **the appropriate inoculum should yield a dense but not completely confluent growth**. It is prepared by diluting an overnight broth culture of the organism in broth. Suitable dilutions of fully grown broth cultures of Enterobacteriaceae and most gram-negative rods are usually about 10^{-4} (1:10,000) while for staphylococci and enterococci a dilution of 10^{-3} (1:1,000) is usually appropriate.
2) The inoculum can also be prepared from isolated colonies on agar. Suspend 2 to 3 colonies into 10 ml physiological saline, and thereafter dilute 0.1 ml/10 ml for Enterobacteriaceae and other gram negative rods or 1 ml/10 ml for staphylococci and enterococci.

Take 1-2 drops (9 cm plate) or 3 to 4 drops (14 cm plate) of the final suspension and apply onto the agar surface and distribute with a bent glassrod or Drigalski spatula. The open plate is then dried at 35-37°C for 10-15 min. before the Neo-Sensitabs are placed onto the agar surface.

When testing slower growing microorganisms, the dilution must be individualized to give semi-confluent growth. The method of inoculation may vary, but it must be such that it gives uniform seeding of the plate and the semi-confluent growth desired.

6.1.2 Inoculum according to CLSI (Kirby-Bauer)

When using the technique of Kirby-Bauer, the inoculum is standardized according to the method described by the CLSI (2), which normally results in more dense growth and therefore smaller inhibition zones than for semiconfluent growth.

1) Growth method:

a) Select at least 3 to 5 isolated colonies. Touch the top of each colony with a wire loop and transfer to a tube containing 4 to 5 ml of a broth medium such as tryptic soy broth.
b) Allow the broth culture to incubate at 35°C until it achieves or exceeds the turbidity of the 0.5 McFarland standard. It usually takes 2 to 6 hours.
c) Adjust the turbidity of the broth culture with sterile saline or broth to obtain a turbidity visually comparable to the standard. This results in a suspension containing approx. 1 to 2 x 10^8 CFU/ml for *E. coli* ATCC 25922.
d) Within 15 minutes, dip a sterile cotton swab into the adjusted suspension and remove inoculum from the swab by exerting firm pressure on the inside of the tube.
e) Inoculate the dried surface of the Mueller-Hinton agar plate by streaking the swab over the entire surface.

2) Direct colony suspension method:

It is also possible to **set up the tests immediately**, rather than wait for a broth sub-culture (3). This can be done by suspending several morphologically similar colonies from an 18-24 h agar plate (non selective) into 4-5 ml 0.9% NaCl solution, and then immediately adjusting the turbidity to match that of the BaSO4 standard (0.5 McFarland).

Within 15 minutes swabs are used to inoculate the test plates. This method was found to be equivalent to the standard CLSI method and requires less time. This method is used in most laboratories for all types of bacteria.

This approach is the recommended for testing the fastidious organisms (*Haemophilus* spp., gonococci, *Moraxella catarrhalis*, pneumococci/streptococci) and for testing staphylococci for potential methicillin or oxacillin resistance (2).

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6.2 Prediffusion Method

Prediffusion Method (2 + 18 hours) for Antimicrobials Diffusing Poorly on Agar

High molecular weight antimicrobials (vancomycin, teicoplanin, daptomycin, colistin) diffuse poorly on agar media, resulting in difficult to interpret results when using the disc diffusion method. Rosco Diagnostica has developed a 2 + 18 hours prediffusion technique, permitting an easier differentiation between susceptible and resistant strains when testing against these antimicrobials.

Procedure

One Neo-Sensitabs of the antimicrobial to be tested is placed on an uninoculated plate containing the susceptibility test medium (Mueller-Hinton plain or BHI Agar + 5 % blood).
After 2 hours at room temperature the tablet (disc) is removed (by knocking the plate agianst the table) and the plate is maintained at room temperature for further 18 hours (overnight).
The plate is now inoculated with the strain to be tested using a McFarland 0.5 inoculum. Additional antibiotic discs (Neo-Sensitabs) may be added now using a dispenser (if MH agar is used) and thereafter the plate is incubated overnight at 35-37 °C.
The zones of inhibition are then measured. Zone breakpoints are tentative and for research use only.

Notice: In the laboratory, the prediffusion plate can be prepared the day before it is inoculated, in which case there is no loss of time and results are obtained within 24 hours.

Interpretation

IA) Detection of Visa/GISA/hVISA strains (medium BHI + 5 % blood), inoculum McF. 0.5

VISA/GISA/hVISA strains will show the following zones of inhibition:

Teicoplanin 30 µg inhibition zone < 20 mm and/or
Vancomycin 5 µg inhibition zone < 22 mm

IB) Detection of GISCN/hGISCN strains (medium BHI + 5 % blood), inoculum McF. 0.5

Teicoplanin 30 µg inhibition zone < 20 mm

GISCN = Glycopeptide intermediate staphylococci, coagulase negative (2).
Strains showing zones of inhibition < 20 mm around Teicoplanin 30 µg should be reported as heteroresistant to both teicoplanin and vancomycin.

IC) Detection of vanA, vanB and vanC in enterococci

Use vancomyicn 5 µg (2+18 h prediffusion), MH agar and McF. 0.5 inoculum.

Susceptible: zone > 16 mm (sharp edge)
VanB: zone < 16 mm (hazy edge)
VanC: zone < 16 mm (sharp edge)

The vanA genotype will show no zone of inhibiton in the current diffusion test with Vancomycin 5 µg Neo-Sensitabs.

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II) Daptomycin testing (medium used Mueller-Hinton plain), inoculum McF. 0.5

a) Staphylococci:

Daptomycin 30 µg Neo-Sensitabs: Susceptible zone ≥ 22 mm (corresponding to an MIC of ≤ 1 µg/ml).

b) Enterococci:

Daptomycin 30 µg Neo-Sensitabs: Susceptible zone ≥ 12 mm (corresponding to an MIC of ≤ 4 µg/ml).

III) Colistin testing (medium used Mueller-Hinton plain), inoculum McF. 0.5

Colistin 10 µg Neo-Sensitabs: Susceptible zone ≥ 15 mm (corresponding to an MIC of ≤ 2 µg/ml).
I: 14-10 mm, R: no zone (MIC ≥ 8 g/ml)

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7 Dispensers and Templates ("Schabelons")

The NEO-SENSITABS Dispensers

The Neo-Sensitabs Dispensers have been designed for uniform and accurate simultaneous positioning of the tablets on an agar plate in any combination desired. The application is one operation, easy, accurate, and time saving.

Today we have 2 different kinds of models available from Rosco:

1) Neo-Sensitabs Dispensers, mobile

These dispensers are to be used with the range of Neo-Sensitabs in **cartridges without a spring**.

Models available:

1) - adaptable to 8-10 cm petri dishes delivering up to 7 Neo-Sensitabs at a time.
2) - for 10 cm petri dishes delivering up to 9 Neo-Sensitabs at a time.
3) - for 14-15 cm petri dishes delivering up to 12 Neo-Sensitabs at a time.
4) - for square petri dishes (12x12 cm) delivering up to 16 Neo-Sensitabs at a time.

The mobile Neo-Sensitabs dispensers are made from transparent acrylic plastic, and the agar surface is plainly visible while the tablets are being transferred.
The tablets come packed in cartridges (tubes), matching the dispenser top-plate holes. Each cartridge accommodates 50 Neo-Sensitabs. For easy identification the bottom of each tube is labelled with a short name (5-digit alpha-numeric code also marked on each tablet) of the antimicrobial contained in the cartridge.

The dispensers ensure uniform tablet location by a pre-determined pattern.
The bottom-plate holes are provided with chutes, ensuring that the tablets are correctly placed onto the agar surface.
The dispensers are equipped with excentrical legs, which are easily turned to make a perfect fit for the petri dishes.
The dispensers may be cleaned with 70 % alcohol, particularly the holes in the top plate, in order to make a good fitting for the cartridges. If one cartridge is found too small, try cleaning of the dispenser top holes to eliminate tablet dust.
A mechanized technique for antibiograms has been described using ROSCO dispensers (2).

2) Dispenser 101 and Dispenser 104 for Neo-Sensitabs

These dispensers are to be used with Rosco Neo-Sensitabs **cartridges with a spring**, where the potency of the tablets are according to recommendations of Clinical and Laboratory Standards Institute (formerly NCCLS) (1).

Models available from Rosco:

1) - Adaptable to 8-10 cm petri dishes delivering up to 7 Neo-Sensitabs at a time.
2) - Adaptable for square petri dishes (12 x 12 cm) delivering up to 16 Neo-Sensitabs at a time.

The Dispenser 101 and Dispenser 104 are made of hard plastic and are operated by pushing the handle down, and the Neo-Sensitabs will be transferred to the agar surface.
When using several dispensers, the colour code on the top of the handles can be used for differentiation.
The holes in the bottom plate ensure that the tablets are placed onto the agar surface in a pre-determined pattern.
The dispensers are easy to disassemble for inside cleaning. They must be cleaned occasionally; wipe with ethanol and hot water to remove dust from the Neo-Sensitabs tablets.

The tablets come packed in cartridges (tubes), matching the dispenser top-plate holes. Each cartridge accommodates 50 Neo-Sensitabs and a red block that prevents dispensing when one cartridge is empty. Insert the cartridges gently and carefully one by one through the top-plates holes. For easy identification the bottom of each tube is labelled with a short name (5-digit alpha-numeric code also marked on each tablet) of the antimicrobial contained in the cartridge.

Further information and Instructions for use is available on www.rosco.dk

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Templates /Schabelons

In order to simplify the interpretation of the zone sizes, a reading chart (template) for Neo-Sensitabs has been devised. The template consists of a plate of transparent acrylic plastic with 7, 9, 12 or 16 holes, corresponding to the dispenser types. Each hole can be filled with a tablet and the corresponding zone size interpretation, in the form of self-adhesive circles, is placed around it.
Currently a template is prepared for each type of test (or dispenser): i.e. enterobacteriaceae from urine, staphylococci from blood, etc.
The template is used by superimposing the susceptibility plate over it, or the template is held on the back of the petri dish illuminated with reflected light.
Templates are made to order according to the individual wishes of each laboratory. Ask the ROSCO representative in your country.

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8 Representative Antimicrobials to be Tested Routinely

Selection of the most appropriate antimicrobials to test and report is a decision made by each laboratory in consultation with the infectious disease practitioners, the pharmacy and infection control committees. Testing of some agents may be useful for infection control or epidemiological purposes (1).
All antimicrobials should be reported by their generic names. To emphasize the relatedness of the currently available drugs, they may be grouped together by drug classes as follows:

The penicillin group of antibiotics:

With regard to antimicrobial effect, it seems appropriate to classify them as:

1) labile to penicillinase (Penicillin G, Penicillin V),
2) stable to penicillinase (Methicillin, Oxacillin),
3) broad-spectrum penicillins (Ampicillin, Amoxycillin),
4) penicillins active against pseudomonas (Ticarcillin),
5) amdino penicillins (Mecillinam),
6) ureido penicillins (Piperacillin),
7) alpha-methoxypenicillins (Temocillin).

1) Penicillin Low Neo-Sensitabs is the representative of the penicillinase labile penicillins (azidocillin, penicillin V, pheniticillin, propicillin, penicillin G). For pneumococci Oxacillin 1 µg Neo-S is used to detect decreased susceptibility to penicillin.
2) Methicillin and Oxacillin Neo-Sensitabs are the representatives of the penicillinase resistant penicillins (cloxacillin, dicloxacillin, flucloxacillin, nafcillin), and when testing against staphylococci, they are also the representatives of all other beta-lactam antibiotics.
3) Ampicillin 33 µg and Amoxycillin Neo-Sensitabs are the representatives of the group including bacampicillin, epicillin, hetacillin, pivampicillin, and talampicillin.
4) Ticarcillin Neo-Sensitabs is the representative of the group including carindacillin and carfecillin (for UTI only).
5) Mecillinam Neo-Sensitabs is the representative of the group including pivmecillinam.
6) Piperacillin Neo-Sensitabs.
7) Temocillin shows a good activity against Enterobacteriaceae and high stability against beta-lactamases.

Beta-lactam/Beta-lactamase inhibitor Combinations:

Amoxycillin+Clavulanate, Ampicillin+Sulbactam, Ticarcillin+Clavulanate and Piperacillin+Tazobactam Neo-Sensitabs, are representatives of combinations of penicillins and betalactamase inhibitors. Ceftazidime+Clavulanate and Cefepime+Clavulanate are useful for detecting ESBLs. Imipenem+EDTA is useful to detect metallo-beta-lactamases (MBL).

Cephalosporins:

1) **First generation of cephalosporins**. Cross-resistance between Cephalothin, Cephalexin, Cefaclor, Cephradine, and Cefadroxil. Cephalothin Neo-Sensitabs is the representative of the group (2). Cefazolin must be tested separately.

2) **Second generation of cephalosporins**. Cefuroxime and Cefonicid have a similar spectrum of activity being active in vitro against some strains resistant to cephalothin. Cephamycins: cefoxitin has a spectrum of activity (including anaerobe strains) unlike other cephalosporins.

3) **Third generation of cephalosporins**. They can be divided into cephalosporins for oral use, including cefixime (broad spectrum), and cephalosporins for injectable use that can be subdivided into two groups:

   A. Narrow spectrum, good activity against pseudomonas: Cefsulodin (non-stock).
   B. Broad spectrum, which may be divided into two subgroups:
      a) Cephamycins, oxa-cephems with good activity against anaerobes and low activity against pseudomonas: Cefotetan.
      b) Moderate activity against anaerobes and b1) good activity against pseudomonas: Ceftazidime, or b2) moderate activity against pseudomonas: Cefotaxime, Ceftriaxone, Ceftizoxime and Cefodizime.

4) **Fourth generation cephalosporins**: Include Cefepime and Cefpirome Neo-Sensitabs.

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Penems and Carbapenems:

Imipenem and Meropenem Neo-Sensitabs have a very broad spectrum of activity and high stability against beta-lactamases. New members of this group are Ertapenem and Doripenem.

Monobactams:

Aztreonam is active only against gram-negative aerobic bacteria.

Macrolides / Streptogramines / Ketolides:

Drugs in the macrolide group (Azithromycin, Clarithromycin, Erythromycin) are closely related and with few exceptions, only Erythromycin may need to be tested routinely.
Clindamycin and Lincomycin have a similar spectrum of activity against aerobes and only one needs to be tested routinely. Against anaerobes clindamycin has a broader spectrum of activity than lincomycin.
Quinupristin/Dalfopristin (Synercid) Neo-Sensitabs and Telithromycin Neo-Sensitabs (ketolid) should be tested separately.

Oxazolidinones:

Linezolid Neo-Sensitabs has activity against gram positive bacteria.

Aminoglycosides:

This class includes members affected by aminoglycoside-inactivating enzymes, which results in some differences in spectrum between the agents. Include Streptomycins 100 µg, Kanamycin 100 µg, Neomycin, Amikacin, Gentamicin 40 µg, Netilmicin, Isepamicin and Tobramycin Neo-Sensitabs. Besides Streptomycins 500 µg, Kanamycin 500 µg and Gentamicin 250 µg Neo-Sensitabs for testing for high level resistance (HLR) with enterococci.

Neomycin, Framycetin, and Paromomycin have a close chemical affinity and only one of them should be tested. (Neomycin Neo-Sensitabs).

Nitroimidazoles:

Cross-resistance between Metronidazole, Ornidazole, and Tinidazole against anaerobes. Only one representative (Metronidazole 16 µg Neo-Sensitabs) needs to be tested routinely.

Polymyxin B and Colistin:

Very closely related and only one needs to be tested routinely. Colistin 10 µg and Polymyxins 150 µg Neo-Sensitabs (colistin).

Quinolones:

Broad spectrum antimicrobials including Ciprofloxacin, Gatifloxacin, Levofloxacin, Moxifloxacin, Norfloxacin, Ofloxacin and Pefloxacin. Small differences in spectrum, may require separate testing of the individual agents.

Sulphonamides:

The Working Group of the W.H.O. expressed the opinion that only one representative sulphonamide needs to be used in sensitivity tests against drugs of the sulphonamide group. (Sulphonamides Neo-Sensitabs) (3).

Tetracyclines:

Most members of this group (oxytetracycline, tetracycline,chlortetracycline) are closely related and for routine work it is enough to test one tetracycline (Tetracyclines 80 µg Neo-Sensitabs) (4). Doxycycline and Minocycline should be tested separately.

Glycopeptides:

Close chemical affinity, but with different activity against some species, Vancomycin and Teicoplanin Neo-Sensitabs, should be tested individually.

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Use of the combination of antibiotics in disks was condemned for the reason stated in the W.H.O. report:
"Diffusion tests employing either a single disk containing two antibiotics at two disks superimposed are useless, since it has been shown that the effect obtained is only that produced by the antibiotic giving the wider zone of inhibition when acting alone".

The combination of trimethoprim and sulphonamides (Trimethoprim+Sulfa Neo-Sensitabs) as well as Amoxycillin+Clavulanate, Ampicillin+Sulbactam, Ticarcillin+Clavulanate, and Piperacillin+Tazobactam are exceptions, because true potentiation or synergism between the two antimicrobials is to be expected in several cases.

Antifungals:

Neo-Sensitabs representing the polyenes, azoles/imidazoles and caspofungin, fluorcytosine for susceptibility testing of yeast are described in this booklet.

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9 Measuring the Inhibition Zones

After incubation at 35°C overnight, the diameter of the zones of inhibition (tablets included) are measured in millimetres using sliding calipers, a ruler, or a template prepared for this purpose, held on the back of the petri dish illuminated with reflected light. If blood has been added to the agar base, the zones should be measured from the surface illuminated with the cover removed.

1) The diameters of the zones of complete inhibition (as judged by the unaided eye) are measured, including the diameter of the tablet/disk (1). Therefore no zone will be registered as 9 mm.

Faint growth of tiny colonies, which can be detected only with a magnifying lens at the edge of the zone of inhibited growth, is ignored. However discrete colonies growing within a clear zone of inhibition should be subcultured, reidentified and retested (resistant mutants or mixed culture).

When the zone limits are clear and sharply outlined, ideal readings can be made.

2) Strains of *Proteus mirabilis* and *Proteus vulgaris* may swarm into areas of inhibited growth. The veil of **swarming** growth **is ignored**.

3) With Chloramphenicol, Erythromycin, and Tetracyclines (bacteriostatic agents) the zones of inhibition will contain a gradient of growth. Zone diameters should be read **halfway** between the start of inhibition and complete inhibition (CLSI 2006).

4) With **Trimethoprim**, **Sulphonamides** and **Trimetroprim+Sulfa** organisms may grow for several generations before being inhibited, resulting in edges of zones of inhibition containing a large number of small colonies. In this case zones of inhibition are measured up to colonies of normal size (disregard slight growth and measure the more obvious margin).

If the test organism is a ***Staphylococcus*** or ***Enterococcus*** **spp.**, **24 hours** of incubation is required and transmitted light (plate held up to light) is used to examine the oxacillin, linezolid and vancomycin zones for **light growth** (minute colonies) of methicillin, linezolid or vancomycin resistant colonies, respectively, within apparent zones of inhibition. Any discernible growth within the zone of inhibition is indicative of methicillin, linezolid or vancomycin-resistance. Other agents can be read after 16-18 hours incubation (1). With staphylococci and linezolid, read with transmitted light and measure only the clear zone (smallest zone).

Serratia marcescens may show a large zone of inhibition around Polymyxins 150 µg (colistin), and within this zone a band of colonies close to the tablet/disk (cocarde). These colonies are polymyxin resistant and consequently *S. marcescens* is to be considered resistant to polymyxins (2).

Brumfitt et al (3) explain the phenomenon of resistance to Amoxycillin+Clavulanate and sensitivity to ampicillin found with a few strains, by the fact that ampicillin in general is more active than amoxycillin against several Entero-bacteriaceae.

The group of strains: *Enterobacter*, *C. freundii*, *Hafnia alvei*, *M. morganii*, *Providencia*, *Proteus* indole positive and *S. marscenscens* all produce an inducible chromosomal AmpC beta-lactamase, which is not inhibited by clavulanate. In most cases, there is an antagonism between amoxycillin and clavulanate (smaller zone with the combination than with amoxycillin alone) due to the presence of the inducible beta-lactamase.
All these strains should be reported as resistant to ampicillin/amoxycillin and to amoxycillin+clavulanate (except *P. vulgaris*).

The presence of **oval zones** is in many cases an indication of heterogeneous cultures, which in most cases are resistant to the particular antimicrobial. Oval zones are measured by taking the mean between the two diameters.

Due to the above mentioned factors, it is not infrequent that, when different persons measure the same zone of inhibition they arrive to discordant results (variations of 2-3 mm are currently seen). It is, therefore, important to establish rules for the reading of inhibition zones. A good procedure is regularly testing of control organisms (see quality control).

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1) NCCLS: Performance Standards for Antimicrobial Disk Susceptibility Tests, 8th Ed. **M2-A8**, January 2003.
2) Annear D.I., Hudson J.A.: An unusual zone surrounding colistin discs in sensitivity tests of Serratia marcescens. The Med. J. Austral. **i**, 840-841, 1970.
3) Brumfitt W. et al: Phenomenon of resistance to Augmentin assosiated with sensitivity to ampicillin: occurrence and explanation. J. Clin. Pathol., **36**, 670-673, 1983.

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10 Zone Diameter Interpretation Tables

10.1 I - Inoculum and MIC Breakpoints according to the CLSI (Kirby-Bauer)

I

CLSI method (Kirby-Bauer) - rapidly growing bacteria Mueller-Hinton agar, inoculum: McFarland 0.5 and break-points according to CLSI (M2-A8)

		NEO-SENSITABS	POTENCY	CODE	Zone diameter in mm S	I	R	Break-points MIC µg/ml S	R
		Amikacin	40 µg	AMIKA	≥ 20	19-17	≤ 16	≤ 16	≥ 32
b)		Amoxycillin*	30 µg	AMOXY	≥ 20	19-17	≤ 16	≤ 8	≥ 32
e)		*Enterococcus* spp.			≥ 20	-	-	-	≥ 16
d)		Amoxycillin+Clav.	30+15 µg	AM+CL	≥ 20	19-17	≤ 16	≤ 8/4	≥ 32/16
		Staphylococcus spp.			≥ 26	-	-	≤ 4/2	≥ 8/4
b)		Ampicillin	33 µg	AMP33	≥ 20	19-17	≤ 16	≤ 8	≥ 32
e)		*Enterococcus* spp.			≥ 20	-	-	-	≥ 16
d)		Ampicillin+Sulbactam	30+30 µg	AM+SU	≥ 20	19-17	≤ 16	≤ 8/4	≥ 32/16
		Azithromycin	30 µg	AZITR	≥ 18	17-15	≤ 14	≤ 2	≥ 8
		Aztreonam	30 µg	AZTRM	≥ 23	22-20	≤ 19	≤ 8	≥ 32
o)		(ESBL screening)			-	-	< 26	-	> 1
		Bacitracin*	40 U	BACIT	≥ 20	19-17	≤ 16	-	-
c)		Cefaclor	30 µg	CCLOR	≥ 20	19-17	≤ 16	≤ 8	≥ 32
c)		Cefadroxil*	30 µg	CFDRO	≥ 20	19-17	≤ 16	≤ 8	≥ 32
c)		Cefazolin	60 µg	CFZOL	≥ 23	22-20	≤ 19	≤ 8	≥ 32
c)		Cefepime	30 µg	CFEPM	≥ 20	19-17	≤ 16	≤ 8	≥ 32
		Cefepime+Clav.	30+10 µg	CP+CL	Detection of ESBL				
c)	m)	Cefixime	30 µg	CFFIX	≥ 26	25-23	≤ 22	≤ 1	≥ 4
c)		Cefonicid	30 µg	CFCID	≥ 20	19-17	≤ 16	≤ 8	≥ 32
c)	o)	Cefotaxime	30 µg	CFTAX	≥ 23	22-20	≤ 19	≤ 8	≥ 32**
c)		Cefotetan	30 µg	CFTTN	≥ 18	17-15	≤ 14	≤ 16	≥ 64
c)		Cefoxitin	60 µg	CFOXT	≥ 23	22-20	≤ 19	≤ 8	≥ 32
t)		*S. aureus* and *S. lugdunensis*			≥ 25	-	≤ 24	Oxa S	Mec A pos.
t)		Coag. neg. staph.			≥ 28	-	≤ 27	Oxa S	Mec A pos.
c)		Cefpirome*	30 µg	CFPIR	≥ 20	19-17	≤ 16	≤ 8	≥ 32
o)		Cefpodoxime	30 µg	CFPOX	≥ 25	24-21	≤ 20	≤ 2	≥ 8
		(ESBL screening)			-	-	< 20	-	> 8
		Cefsulodin*	30 µg	CFSUL	≥ 23	22-20	≤ 19	≤ 8	≥ 32
c)		Ceftazidime	30 µg	CEZDI	≥ 20	19-17	≤ 16	≤ 8	≥ 32
o)		(ESBL screening)			-	-	< 24	-	> 2
		Ceftazidime+Clav.	30+10 µg	CZ+CL	Detection of ESBL				
c)		Ceftizoxime	30 µg	CEZOX	≥ 23	22-20	≤ 19	≤ 8	≥ 32
		S. aureus			≥ 23	-	≤ 20	Oxa S	Mec A pos. (Oxa R)
c)		Ceftriaxone	30 µg	CETRX	≥ 20	19-17	≤ 16	≤ 8	≥ 32**
o)		(ESBL screening)			-	-	< 24	-	> 2
c)	k)	Cefuroxime (parenteral)	60 µg	CEFUR	≥ 23	22-20	≤ 19	≤ 8	≥ 32
c)	k)	Cefuroxime (oral)	60 µg	CEFUR	≥ 25	24-21	≤ 20	≤ 4	≥ 32
c)		Cephalexin*	30 µg	CFLEX	≥ 20	19-17	≤ 16	≤ 8	≥ 32

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	NEO-SENSITABS	POTENCY	CODE	Zone diameter in mm S	I	R	Break-points MIC µg/ml S	R
c)	Cephalothin	66 µg	CLOTN	≥ 23	22-20	≤ 19	≤ 8	≥ 32
c)	Cephradine*	60 µg	CFRAD	≥ 23	22-20	≤ 19	≤ 8	≥ 32
	Chloramphenicol	60 µg	CLR60	≥ 25	24-21	≤ 20	≤ 8	≥ 32
a)	Cinoxacin (U)	30 µg	CINOX	≥ 16	15-14	≤ 13	≤ 16	≥ 64
q)	Ciprofloxacin	10 µg	CIP10	≥ 20	19-17	≤ 16	≤ 1	≥ 4
	Ciprofloxacin	0.5 µg	-----					
	Salmonella spp.			≥ 24	-	≤ 23	≤ 0.06	≥ 0.12
	Clarithromycin	30 µg	CLARI	≥ 18	17-15	≤ 14	≤ 2	≥ 8
j)	Clindamycin	25 µg	CLIND	≥ 26	25-23	≤ 22	≤ 0.5	≥ 4
	Cloxacillin	500 µg	CL500	Detection of plasmid mediated AmpC beta-lactamases				
s)	Colistin	10 µg	CO.10					
	2+18 h prediffusion			≥ 15	14-11	≤ 10	≤ 2	≥ 8
v)	Daptomycin	30 µg	DAPCa					
	Staphylococcus spp. 2+18 h prediffusion			≥ 22	-	-	≤ 1	-
	Enterococcus faecalis (Vanco S) 2+18h pred.			≥ 12	-	-	≤ 4	-
	Doxycycline	80 µg	DOXYC	≥ 20	19-17	≤ 16	≤ 4	≥ 16
q)	Enrofloxacin (Vet.)	10 µg	ENROF	≥ 23	22-17	≤ 16	≤ 0.5	≥ 4
c)	Ertapenem	10 µg	ERTAP	≥ 19	18-16	≤ 15	≤ 2	≥ 8
j)	Erythromycin	78 µg	ERYTR	≥ 26	25-19	≤ 18	≤ 0.5	≥ 8
	Flumequine* (Vet.)	30 µg	FLUME	≥ 20	19-17	≤ 16		
a) l)	Fosfomycin (U)	70+40 µg	FOSFO	≥ 16	15-14	≤ 13	≤ 64	≥ 256
i)	Fucidin*	100 µg	FUCID	≥ 28	27-24	≤ 23	≤ 1	≥ 4
	Furazolidone*	50 µg	FURAZ	≥ 23	22-20	≤ 19	≤ 4	≥ 8
q)	Gatifloxacin	5 µg	GATIF	≥ 18	17-15	≤ 14	≤ 2	≥ 8
	Staphylococcus spp.			≥ 23	22-20	≤ 19	≤ 0.5	≥ 2
r)	Gentamicin	**40 µg**	GEN40	≥ 23	22-20	≤ 19	≤ 4	≥ 8
f)	Gentamicin (Enterococci HLR all amino glycosides)	**250 µg**	GN250	-	-	< 14	-	> 500
c)	Imipenem	15 µg	IMIPM	≥ 20	19-17	≤ 16	≤ 4	≥ 16
	Imipenem+EDTA	15+750 µg	IM+ED	Detection of metallo-ß-lactamases				
	Isepamicin*	30 µg		≥ 20	19-17	≤ 16	≤ 8	≥ 32
	Kanamycin	**100 µg**	KANAM	≥ 25	24-21	≤ 20	≤ 6	≥ 25
f)	Kanamycin (Enterococci HLR amikacin)	**500 µg**	KA500	-	-	< 14	-	> 1000
q)	Levofloxacin	5 µg	LEVOF	≥ 16	15-14	≤ 13	≤ 2	≥ 8
	Staphylococcus spp.			≥ 19	18-16	≤ 15	≤ 1	≥ 4
	Lincomycin	19 µg	LINCO	≥ 26	25-23	≤ 22	≤ 2	≥ 8
	Linezolid	30 µg	LINEZ					
	Enterococcus spp.			≥ 23	22-21	≤ 20	≤ 2	≥ 8
	Staphylococcus spp.			≥ 21	-	≤ 20	≤ 4	-
a) c)	Mecillinam (U)	33 µg	MECIL	≥ 18	17-15	≤ 14	≤ 8	≥ 32
c)	Meropenem	10 µg	MEROP	≥ 18	17-15	≤ 14	≤ 4	≥ 16
x)	*Pseudomonas* spp.			≥ 26	25-21	≤ 20	≤ 4	≥ 16
c)	Methicillin	29 µg	METHI	≥ 20	19-17	≤ 16	≤ 8	≥ 16
	Minocycline	80 µg	MINOC	≥ 22	21-19	≤ 18	≤ 4	≥ 16
q)	Moxifloxacin	5 µg	MOXIF	≥ 19	18-16	≤ 15	≤ 2	≥ 8
	Staphylococcus spp.			≥ 24	23-21	≤ 20	≤ 0.5	≥ 2
	Mupirocin*	10 µg	MUPIR	≥ 14	-	≤ 13	≤ 4	≥ 8

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	NEO-SENSITABS	POTENCY	CODE	Zone diameter in mm S	I	R	Break-points MIC µg/ml S	R
a) q)	Nalidixan (U)	130 µg	NALID	≥ 25	24-21	≤ 20	≤ 8	≥ 32
	Enterobacteriaceae			-	-	< 25	Reduced susceptibility to quinolones	
	Neomycin*	120 µg	NEOMY	≥ 25	24-21	≤ 20	≤ 6	≥ 25
	Netilmicin	40 µg	NETIL	≥ 20	19-17	≤ 16	≤ 12	≥ 32
a)	Nitrofurantoin (U)	260 µg	NITRO	≥ 23	22-20	≤ 19	≤ 32	≥ 128
a) q)	Norfloxacin (U)	10 µg	NORFX	≥ 16	15-14	≤ 13	≤ 4	≥ 16
i) *)	Novobiocin	**5 µg**	NOVO5	≥ 16	15-14	≤ 13	≤ 2	-
q)	Ofloxacin	10 µg	OFLOX	≥ 18	17-15	≤ 14	≤ 2	≥ 8
	Staphylococcus spp.			≥ 20	19-17	≤ 16	≤ 1	≥ 4
c)	Oxacillin	**1 µg**	OXA.1					
	S. aureus			≥ 13	12-11	≤ 10	≤ 2	≥ 4
	Coag. neg. staph.			≥ 18	-	≤ 17	≤ 0.25	≥ 0.5
c)	Oxacillin	**5 µg**	OXA.5					
	S. aureus			≥ 16	15-14	≤ 13	≤ 2	-
a)	Oxolinic acid* (U)	10 µg	OXOLI	≥ 16	15-14	≤ 13	≤ 4	≥ 8
q)	Pefloxacin*	10 µg	PEFLX	≥ 18	17-15	≤ 14	≤ 2	≥ 8
	Penicillin Low	5 µg	PEN.L	≥ 26	25-23	≤ 22		
b)	*Staphylococcus* spp.			≥ 26	-	< 26	≤ 0.1	Beta-Lactamase
e)	*Enterococcus* spp.			≥ 10	-	-	-	≥ 16
a)	Pipemidic acid* (U)	30 µg	PIPEM	≥ 20	19-17	≤ 16	≤ 4	≥ 16
b)	Piperacillin	100 µg	PIPRA	≥ 23	22-20	≤ 19	≤ 16	≥ 128
h)	*Pseudomonas* spp.			≥ 18	-	-	≤ 64	≥ 128
d)	Piperacillin+Tazobactam	100+10µg	PI+TZ	≥ 23	22-20	≤ 19	≤ 16/4	≥ 128/4
h)	*Pseudomonas* spp.			≥ 18	-	-	≤ 64/4	≥ 128/4
s)	Polymyxins* (colistin)	150 µg	CO150	≥ 20	19-17	≤ 16	≤ 2	≥ 4
	Pristinamycin*	30 µg	PRIST	≥ 23	22-20	≤ 19	≤ 2	≥ 8
	Quinupristin/Dalfopristin	15 µg	SYN15	≥ 19	18-16	≤ 15	≤ 1	≥ 4
	Rifampicin	30 µg	RIFAM	≥ 26	25-23	≤ 22	≤ 1	≥ 4
	P. aeruginosa			≥ 23	22-18	≤ 17	≤ 4	≥ 16
q)	Sparfloxacin*	10 µg		≥ 20	19-17	≤ 16	≤ 1	≥ 4
	Spectinomycin (not gonococci)	200 µg	SPECT	≥ 20	19-17	≤ 16	≤ 16	≥ 64
	Spiramycin*	200 µg	SPIRA	≥ 26	25-23	≤ 22	≤ 2	≥ 8
	Streptomycin	100 µg	ST100	≥ 26	25-23	≤ 22	≤ 6	≥ 25
f)	Streptomycin (*Enterococcus* spp. HLR)	500 µg	ST500	-	-	< 14	-	> 1000
a)	Sulphonamides (U)	240 µg	SULFA	≥ 23	22-20	≤ 19	≤ 100	≥ 350
	Teicoplanin	30 µg	TPN30	≥ 14	13-11	≤ 10	≤ 8	≥ 32
v)	2+18 h prediffusion (staph.)			-	-	< 20	-	GISA/TRCNS
	Telithromycin	15 µg	TEL15	≥ 22	21-19	≤ 18	≤ 1	≥ 4
	Temocillin*	30 µg	TEMOC	≥ 18	17-15	≤ 14	≤ 16	≥ 32
	Tetracyclines	80 µg	TET80	≥ 22	21-19	≤ 18	≤ 4	≥ 16
b)	Ticarcillin	75 µg	TICAR	≥ 20	19-17	≤ 16	≤ 16	≥ 128
h)	*Pseudomonas* spp.			≥ 16	-	-	≤ 64	≥ 128
d)	Ticarcillin+Clavulanate	75+15 µg	TI+CL	≥ 23	22-20	≤ 19	≤ 16/2	≥ 128/2
h)	*Pseudomonas* spp.			≥ 16	-	-	≤ 64/2	≥ 128/2
u)	Tigecycline	15 µg	TIG15					
	Enterobacteriaceae			≥ 19	18-15	≤ 14	≤ 2	≥ 8
	Staphylococcus spp.			≥ 19	-	-	≤ 0.5	-
	Enterococcus spp.			≥ 19	-	-	≤ 0.25	-

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	NEO-SENSITABS	POTENCY	CODE	Zone diameter in mm S	I	R	Break-points MIC µg/ml S	R
	Tobramycin	40 µg	TOBRA	≥ 23	22-20	≤ 19	≤ 4	≥ 8
	Trimethoprim (U)	5.2 µg	TRIME	≥ 20	19-17	≤ 16	≤ 4	≥ 16
	Trimethoprim+Sulfa	5.2+240 µg	TR+SU	≥ 28	27-24	≤ 23	≤ 2/38	≥ 8/152
g)	Vancomycin	5 µg	VAN.5	≥ 15	14-13	≤ 12	≤ 4	≥ 32
	S. aureus			≥ 16	15-13	≤ 12	≤ 2	≥ 16
v)	2+18 hours' prediffusion *** (staph.)			-	-	< 22	-	hVISA/VISA
	2+18 hours' prediffusion (enterococci)			-	-	< 16	-	VRE
	Virginiamycin*	30 µg	VIRGI	≥ 23	22-20	≤ 19	≤ 2	≥ 6

*) Break-points have not been established by the CLSI (M2-A8 (M100-S14)).

**) We recommend 32 µg/ml as break-point for resistance because we believe that some resistant strains may be falsely recorded as "Intermediate" when using the 64 µg/ml break-point recommended by the CLSI.

***) See description of this technique on page 86.

Remarks:

a) For urinary tract infections only.

b) Staphylococci resistant to Penicillin Low (beta-lactamase producers) should be reported as resistant to penicillin G, penicillin V, amoxycillin, ampicillin, azlocillin, carbenicillin, piperacillin, and ticarcillin.

c) **Cefoxitin** is preferable to detect **methicillin resistance in staphylococci** because it is more likely to detect heteroresistance. Strains that are resistant to **Cefoxitin Neo-S** should be be reported resistant to *all* other beta-lactams: penicillins, beta-lactamase inhibitor combinations, cephalosporins, and carbapenems.

d) **Amoxycillin+Clavulanate** and **Ampicillin+Sulbactam**: For **staphylococci** it is preferable to use **Cefoxitin**. Staphylococci resistant to Oxacillin/Methicillin should be reported resistant to all combinations with beta-lactamase inhibitors.

e) **Enterococci**: For treatment of serious infections **S** refers to high-dose combination therapy.

f) When testing **enterococci** use **Gentamicin 250 µg** and **Streptomycin 500 µg** to detect high-level resistance **(HLR)**. Kanamycin 500 µg can be used to detect high-level resistance to amikacin. Strains that are HLR to gentamicin are HLR to *all* aminoglycosides (including amikacin) except streptomycin.

g) When testing enterococci and staphylococci, plates should be incubated for **full 24 hours** and examined carefully for the presence of a haze or other growth within the zone (indicates resistance) (see chapter 14.2).
CLSI recommend a reference MIC method for staphylococcal isolates showing inhibition zones smaller than the limit for susceptible (chapter 13)

h) ***Pseudomonas aeruginosa***. Serious infections should be treated with maximum doses of antipseudomonal penicillin or ceftazidine, in combination with an aminoglycoside. According to CLSI: The susceptibility of *Ps. aeruginosa* isolated from patients with cystic fibrosis can be reliably determined by the diffusion method, but may require extended incubation up to 24 hours (CLSI M100-S17, 2007).

i) When **blood** is added to the medium the interpretation is: **Novobiocin 5 µg**: **S:** > 13 mm; **I:** 12-11 mm; **R:** < 10 mm, and **Fucidin**: **S:** > 26 mm; **I:** 25-23 mm; **R:** < 22 mm. Addition of blood should only be used when the strain does not grow well on unsupplemented Mueller-Hinton agar.

j) For the detection of Erythromycin resistance phenotypes use the double tablet induction test (see chapter 13.3, page 89).

k) **Cefuroxime** Neo-Sensitabs is used to test both **cefuroxime sodium** (injectable) (23/19) and **cefuroxime axetil** (oral) (25/20). The different zone sizes correspond to the recommended MIC break-points.

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l) **Fosfomycin**: Indicated for use against *E. coli* and *Ent. faecalis* only (M100-S15, 2005).

m) **Morganella morganii** should not be tested against **Cefixime** because false susceptible results may occur.

n) All staphylococci with zone diameters < 17 mm with Teicoplanin Neo-S, should be tested using the 2+18 hours' prediffusion test with Vancomycin 5 µg and Teicoplanin 30 µg (page 86 and page 18).

o) Strains of Klebsiella spp., Salmonella and E. coli that **produce ESBL** may be clinically resistant to therapy with penicillins, cephalosporins or aztreonam, despite apparent in vitro susceptibility (M100-S17, 2007). See chapter 16.1 for ESBL screening and confirmatory tests.

q) Enterobacteriaceae susceptible to nalidixic acid (NALI) are susceptible to quinolones (MIC ciprofloxacin ≤ 0.06 µg/ml). Strains resistant to NALI (zone < 25 mm) show a **decreased** susceptibility to quinolones (MIC CIPRO ≥ 0.125 µg/ml). Therefore Nalidixic acid is a good screening for the detection of decreased fluoroquinolone susceptibility in *Salmonella* spp. (Hakanen A. et al: J. Clin. Microbiol. 37, 3572-77, 1999) that may be associated with clinical failure (CLSI 2003).
According to Hakanen et al. (JCM **43**, 577-8, 2005) *Salmonella enterica* isolates from Southeast Asia may show a new quinolone resistance pattern: NALI susceptible and CIPRO reduced susceptibility (MIC 0.12-0.25 µg/ml). Therefore test both NALI and Ciprofloxacin 0.5 µg Neo-Sensitabs.

r) Staphylococci that are **resistant** to gentamicin, should be reported as resistant against both netilmicin and tobramycin (enzymes APH(2")+AAC(6')).

s) Rosco Diagnostica has developed a 2+18 hours' prediffusion technique for colistin permitting a clear differentiation between susceptible and resistant strains (see page 18). Colistin 10 µg disk testing without prediffusion can be used as screening test for high level resistance with *P. aeruginosa* (MIC ≥ 128 µg/ml corresponds to no zone of inhibition).

t) Cefoxitin should be used to detect MRSA (heterogeneous resistance). Cefoxitin Neo-Sensitabs: S ≥ 25 mm (methicillin susceptible) and R ≤ 24 mm (methicillin resistant, mecA positive). For coag. neg. staph. except *S. lugdunensis* use: S ≥ 28 mm and R ≤ 27 mm. For *S. aureus* incubate for 18-24 h. For coagulase negative staphylococci incubate for 24 h. Results may be reported at 18 h if resistant.

u) Tentative FDA breakpoints (September 2005).

v) A 2+18 hours' prediffusion technique has been developed by Rosco Diagnostica. Zone breakpoints are tentative for 1 year. Description of the technique on page 18.

x) *P. aeruginosa* low level resistance to meropenem (MIC 8 µg/ml) cannot be detected by the interpretation recommended by the CLSI. Therefore, we recommend using Rosco's own interpretation: S ≥ 22; R < 18 mm.

Note: For fecal isolates of **Salmonella** and ***Shigella* spp.**, only ampicillin, a quinolone (nalidixic acid), and trimethoprim + sulfa should be tested and reported routinely. In addition, chloramphenicol and a third generation cephalosporin should be tested and reported for extraintestinal isolates of *Salmonella* spp. Aminoglycosides as well as first and second gen. cephalosporins are not effective clinically (may appear active in vitro).

- Second and third generation cephalosporins are associated with emergence of resistance during prolonged therapy for **Enterobacter**, Citrobacter and Serratia. Susceptible isolates may become resistant within a few days after initiation of therapy.

- ***Pseudomonas aeruginosa*** may develop resistance during prolonged therapy with all antimicrobials (testing of repeat isolates). The susceptibility of *Ps. aeruginosa* isolated from patients with cystic fibrosis can be reliably determined by the diffusion method, but may require incubation up to 24 hours (CLSI 2001).

- For Enterobacteriacea isolated from the **CSF test cefotaxime** (or ceftriaxone) instead of cephalothin (or cefazolin).

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10.2 II - Interpretations according to MIC Breakpoints of the Dutch CRG

II-a

Susceptibility testing of rapidly growing bacteria using Neo-Sensitabs and MIC break-points as recommended by the Dutch CRG (September 2000)
Medium Iso-Sensitest. Inoculum according to ICS

	NEO-SENSITABS	POTENCY	CODE	Zone diameter in mm S	I	R	Break-points MIC µg/ml S	R
	Ampicillin	33 µg	AMP33	≥ 28	27-18	< 18	≤ 2	> 16
	Amoxycillin	30 µg	AMOXY	≥ 28	27-18	< 18	≤ 2	> 16
a)	Amoxycillin+Clav.	30+15 µg	AM+CL	≥ 28	27-18	< 18	≤ 2+0.5	> 16+4
b)	Methicillin	29 µg	METHI	-	-	-	≤ 4	> 4
b)	Oxacillin (MH, McF 0.5)	1 µg	OXA.1					
	S. aureus			≥ 13	-	< 13	≤ 2	> 2
	Staphylococcus spp.			≥ 18	-	< 18	≤ 0.25	≥ 0.5
c)	Penicillin Low	5 µg	PEN.L					
	Staphylococcus spp.			≥ 28	-	< 28	≤ 0.25	Beta-Lactamase
	Others			≥ 28	27-10	< 10	≤ 0.25	> 4
	Piperacillin	100 µg	PIPRA	≥ 26	25-20	< 20	≤ 16	> 64
	Piperacillin+Tazobactam	100+10µg	PI+TZ	≥ 26	25-22	< 22	≤ 16+2	> 32+4
	Temocillin	30 µg	TEMOC	≥ 20	19-15	< 15	≤ 8	> 32
	Ticarcillin	75 µg	TICAR	≥ 26	25-20	< 20	≤ 16	> 64
	Ticarcillin+Clavulanate	75+15 µg	TI+CL	≥ 26	25-20	< 20	≤ 16+4	> 64+4
d)	Cefaclor	75+15 µg	TI+CL	≥ 26	25-20	< 20	≤ 4	> 16
	Cefazolin	60 µg	CFZOL	≥ 28	27-23	< 23	≤ 4	> 16
e) m)	Cefotaxime	30 µg	CFTAX	≥ 26	25-20	< 20	≤ 4	> 16
	Cefotetan	30 µg	CFTTN	≥ 22	21-18	< 18	≤ 4	> 16
b_2)	Cefoxitin	60 µg	CFOXT	≥ 28	27-23	< 23	≤ 4	> 16
	S. aureus and *S. lugdunensis*			≥ 30	29-28	< 28	Oxa S	mec A pos.
	Coag. neg. staph.			≥ 34	33-32	< 32	Oxa S	mec A pos.
b_2)	Cefoxitin	10 µg	CFO10					
	S. aureus and *S. lugdunensis*			≥ 22	-	< 22	Oxa S	mec A pos.
	Coag. neg. staph.			≥ 27	26-22	< 22	Oxa S	mec A pos.
	Cefsulodin	30 µg	CFSUL	≥ 26	25-20	< 20	≤ 4	≥ 16
e) m)	Ceftazidime	30 µg	CEZDI	≥ 26	25-20	< 20	≤ 4	> 16
e) m)	Ceftriaxone	30 µg	CETRX	≥ 26	25-20	< 20	≤ 4	> 16
	Cefuroxime (parenteral)	60 µg	CEFUR	≥ 28	27-23	< 23	≤ 4	> 16
d)	Cefuroxime (oral)	60 µg	CEFUR	≥ 34	33-23	< 23	≤ 1	> 16
d)	Cephalexin	30 µg	CFLEX	≥ 26	25-20	< 20	≤ 4	> 16
	Cephalotin	66 µg	CLOTN	≥ 28	27-23	< 23	≤ 4	> 16
d)	Cephadrine	60 µg	CFRAD	≥ 26	25-20	< 20	≤ 4	> 16
	Cefixime	30 µg	CFFIX	≥ 30	29-26	< 26	≤ 1	> 2
	Cefepime	30 µg	CFEPM	≥ 26	25-20	< 20	≤ 4	> 16
	Cefpodoxime	30 µg	CFPOX	≥ 32	31-30	< 30	≤ 0.25	> 0.5
e) m)	Aztreonam	30 µg	AZTRM	≥ 26	25-22	< 22	≤ 4	> 8
l)	Imipenem	15 µg	IMIPM	≥ 28	27-20	< 20	≤ 2	> 8
l)	Meropenem	10 µg	MEROP	≥ 26	25-20	< 20	≤ 2	> 8

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NEO-SENSITABS		POTENCY	CODE	Zone diameter in mm S	I	R	Break-points MIC µg/ml S	R
f)	Amikacin	40 µg	AMIKA	≥ 26	25-22	< 22	≤ 4	> 16
f)	Gentamicin	**40 µg**	GEN40	≥ 30	29-23	< 23	≤ 1	> 4
f)	Gentamicin	**250 µg**	GN250	-	-	< 16	-	> 1000 (HLR)
f) g)	Kanamycin	**100 µg**	KANAM	≥ 28	27-23	< 23	≤ 4	> 16
f)	Kanamycin	**500 µg**	KA500	-	-	< 16	-	> 1000 (HLR)
f)	Netilmicin	40 µg	NETIL	≥ 28	27-23	< 23	≤ 2	> 8
f) g)	Streptomycin	**100 µg**	ST100	≥ 30	29-23	< 23	≤ 4	> 16
f)	Streptomycin	**500 µg**	ST500	-	-	< 16	-	> 1000 (HLR)
f)	Tobramycin	40 µg	TOBRA	≥ 30	29-23	< 23	≤ 1	> 4
	Neomycin	120 µg	NEOMY	≥ 26	25-20	< 20	≤ 4	> 8
h)	Ciprofloxacin	10 µg	CIP10	≥ 24	23-20	< 20	≤ 1	> 2
h)	Norfloxacin (U)	10 µg	NORFX	≥ 24	23-20	< 20	≤ 1	> 2
h)	Ofloxacin	10 µg	OFLOX	≥ 24	23-18	< 18	≤ 1	> 4
h)	Levofloxacin	5 µg	LEVOF	≥ 26	25-18	< 18	≤ 0.5	> 4
h)	Pefloxacin	10 µg	PEFLX	≥ 24	23-18	< 18	≤ 1	> 4
	Pipemidic acid (U)	30 µg	PIPEM	≥ 20	-	< 20	≤ 8	> 8
j)	Nalidixan (U)	130 µg	NALID	≥ 28	27-23	< 23	≤ 8	> 8
	Cinoxacin (U)	30 µg	CINOX	≥ 22	-	< 22	≤ 8	> 8
	Bacitracin	40 U	BACIT	≥ 22	21-18	< 18	-	-
	Clarithromycin	30 µg	CLARI	≥ 24	23-18	< 18	≤ 0.5	> 2
	Erythromycin	78 µg	ERYTR	≥ 28	27-23	< 23	≤ 1	> 2
	Linezolid	30 µg	LINEZ	≥ 28	27-23	< 23	≤ 2	> 8
	Fosfomycin	70+40 µg	FOSFO	≥ 28	27-23	< 23	≤ 16	> 32
	Chloramphenicol	60 µg	CLR60	≥ 28	27-23	< 23	≤ 4	> 8
	Clindamycin	25 µg	CLIND	≥ 26	25-20	< 20	≤ 1	> 4
	Quinupristin/Dalfopristin	15 µg	SYN15	≥ 20	19-16	< 16	≤ 1	≥ 4
	Fucidin	100 µg	FUCID	≥ 32	-	< 32	≤ 1	> 1
	Nitrofurantoin (U)	260 µg	NITRO	≥ 24	-	< 24	≤ 32	> 32
	Polymyxins	150 µg	CO150	≥ 22	21-18	< 18	≤ 4	> 8
	Rifampicin	30 µg	RIFAM	≥ 28	-	< 28	≤ 1	> 1
g)	Sulphonamides	240 µg	SULFA	≥ 30	29-23	< 23	≤ 32	> 64
	Teicoplanin	30 µg	TPN30	≥ 16	15-13	< 13	≤ 2	> 4
	Tetracyclines	80 µg	TET80	≥ 28	27-23	< 23	≤ 1	> 4
	Doxycycline	80 µg	DOXYC	≥ 28	27-23	< 23	≤ 1	> 4
	Trimethoprim (U)	5.2 µg	TRIME	≥ 22	21-18	< 18	≤ 1	> 2
g)	Trimethoprim+Sulfa	5.2+240 µg	TR+SU	≥ 28	27-23	< 23	≤ 1+19	> 2+38
i) n)	Vancomycin	5 µg	VAN.5	≥ 15	14-13	< 13	≤ 4	> 8
	Enterococcus spp.			≥ 17	16-14	< 14	≤ 4	> 8
i)	*Enterococcus* spp. (McF 0.5)			≥ 15	14-13	< 13	≤ 4	> 4

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Remarks:

a) **Amoxycillin+Clavulanate**: Tentative zone limits until further testing has been completed. For staphylococci test Oxacillin 1 µg (see b).

b) **Methicillin**: The MIC-breakpoint is valid only for **staphylococci** and refers to MICs performed on Mueller-Hinton agar at 30 °C.

Methicillin resistance in staphylococci is best detected using Mueller-Hinton agar, inoculum equivalent to McFarland 0.5 (10^8 CFU/ml), **Oxacillin 1µg** and incubation at 30-35 °C for full 24 hours for *S. aureus* **(S: ≥ 13 mm; R: < 13 mm)** and 48 hours for coagulase-negative staphylococci **(S: ≥ 18 mm; R: < 18 mm)**. Staphylococci that are resistant to Oxacillin 1 µg (methicillin) should also be considered as resistant to all other beta-lactams: penicillins, beta-lactamase inhibitor combinations, cephalosporins, and carbapenems.

b_2) Cefoxitin is now the best test to detect mecA positive staphylococci.

c) **Staphylococci** resistant to **Penicillin Low** should also be considered as resistant to amoxycillin, ampicillin, piperacillin and ticarcillin.

d) The relatively high MIC-breakpoint for I/R for the oral cephalosporins is valid only for urinary tract infections.

e) When testing **Pseudomonas** with **Cefotaxime**, **Ceftazidime**, **Ceftriaxone**,and **Aztreonam** use: **S: > 30 mm; I: 29-23 mm; R: < 23 mm**.

f) When testing **enterococci** use **Gentamicin 250 µg** and **Streptomycin 500** µg to detect high-level resistance **(HLR < 16 mm)**. Kanamycin 500 µg can be used to detect highlevel resistance to amikacin (HLR < 16 mm). Strains that are HLR to gentamicin are HLR to all aminoglycosides (including amikacin) except streptomycin.

g) When testing **Pseudomonas** with **Kanamycin 100 µg**, **Streptomycins 100 µg**, **Sulphonamides**, and **Trimethoprim+Sulfa** use: **S: 32 mm; I: 31-28 mm; R: < 28 mm**.

h) When testing **Pseudomonas** with **Ciprofloxacin 10 µg**, **Norfloxacin**, **Levofloxacin**, **Ofloxacin**, and **Pefloxacin** use: **S: > 28 mm; I: 27-23 mm; R: < 23 mm**.

i) When testing **enterococci** use **Vancomycin 5 µg**. Plates should be incubated **full 24 hours** and examined carefully for the presence of a haze or other growth within the zone (indicates resistance). It is preferable to use a heavier inoculum equivalent to McFarland 0.5 **(S: > 15 mm; I: 14-13 mm; R: < 12 mm)**.

j) Nalidixan is a good screening for the detection of decreased fluoroquinolone susceptibility in Salmonella spp. (Hakanen A. et al: J. Clin. Microbiol. 37, 3572-77, 1999). Strains resistant to Nalidixan Neo-S (zone < 28 mm) show a decreased susceptibility to quinolones (ciprofloxacin MIC > 0.125 µg/ml).

l) When testing **Pseudomonas** use S > 30 mm, I: 29-23 mm, R < 23 mm. Carbapenem testing on Iso-Sensitest agar may give false susceptibility results for isolates that may harbour metallo-beta-lactamases; testing on Mueller Hinton agar may be preferred (BSAC 2001).

m) Strains of Klebsiella, Salmonella and *E.coli* that produce ESBL may be clinically resistant to therapy with penicillins, cephalosporins or aztreonam, despite apparent in vitro susceptibility. See chapter 16.1 for ESBL screening and confirmatory tests.

n) For the detection of staphylococci with reduced susceptibility to vancomycin/teicoplanin (VISA, GISA hVISA) see the chapter in User's Guide on detection of strains with decreased susceptibility to vancomycin page 86.

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II-b

Haemophilus spp., *S. pneumoniae*, *N. gonorrhoeae*, *N. meningitidis* and *H. pylori*. Interpretation according to the MIC break-points recommended by the Dutch CRG (2000) Inoculum McFarland 0.5 or 3 (*H. pylori*)

				Zone diameter in mm			Break-points MIC µg/ml	
	NEO-SENSITABS	POTENCY	CODE	S	I	R	S	R
	Penicillin Low	5 µg	PEN.L					
c)	*S. pneumoniae*			(Use Oxacillin 1 µg)				
g)	*N. gonorrhoeae*			≥ 34	33-22	< 22	≤ 0.06	> 1
	N. meningitidis			≥ 26	25-22	< 22	≤ 0.06	> 1
	Ampicillin	2.5 µg	AMP.L					
a)	*Haemophilus* spp.			≥ 22	21-18	< 18	≤ 0.5	> 2
	Amoxycillin	30 µg	AMOXY					
a)	*Haemophilus* spp.			≥ 28	27-24	< 24	≤ 1	> 2
	Amoxycillin+Clav.	30+15 µg	AM+CL					
a)	*Haemophilus* spp.			≥ 28	27-24	< 24	≤ 1/0.25	> 2/0.5
	Oxacillin	1 µg	OXA.1					
c)	*S. pneumoniae*			≥ 20	< 20	< 20	≤0.06 (pen)	MIC
h)	*N. gonorrhoeae*			≥ 12	(pen. screening)	-	≤0.06 (pen)	-
i)	*N. meningitidis*			≥ 10	(pen. screening)	-	≤0.06 (pen)	MIC
	Cefuroxime	60 µg	CEFUR					
a)	*Haemophilus* spp.			≥ 30	29-26	< 26	≤ 1	> 2
	N. gonorrhoeae			≥ 36	-	< 36	≤ 0.25	-
	Cefotaxime	30 µg	CFTAX					
d)	*S. pneumoniae*			(Use Ceftizoxime)				
	N. gonorrhoeae			≥ 32	-	-	≤ 0.25	-
	N. meningitidis			≥ 32	31-28	< 28	≤ 0.5	> 1
	Ceftriaxone	30 µg	CETRX					
d)	*S. pneumoniae*			(Use Ceftizoxime)				
	N. gonorrhoeae			≥ 32	-	-	≤ 0.25	-
	N. meningitidis			≥ 32	31-28	< 28	≤ 0.5	> 1
	Ceftizoxime	30 µg	CEZOX					
d)	*S. pneumoniae*			≥ 30	-	< 30	≤ 0.5 3rd gen.cepha.	MIC
	Imipenem	15 µg	IMIPM					
	S. pneumoniae			≥ 34	33-30	< 30	≤ 0.12	> 0.5
	Azithromycin	30 µg	AZITR					
	Campylobacter spp.			≥ 23	22-19	< 19	≤ 1	> 2
	Erythromycin	78 µg	ERYTR					
e)	*S. pneumoniae*			≥ 28	27-25	< 25	≤ 0.25	> 0.5
	Clarithromycin	30 µg	CLARI					
	H. pylori			≥ 28	27-24	< 24	≤ 0.25	> 1
b)	Ciprofloxacin	**0.5 µg**	CIP.L					
f)	*N. gonorrhoeae*			≥ 20	19-15	< 15	≤ 0.06	> 1
b)	Ciprofloxacin	**10 µg**	CIP10					
f)	*N. gonorrhoeae*			≥ 36	35-30	< 30	≤ 0.06	> 1
	Tetracyclines	80 µg	TET80					
	N. gonorrhoeae			≥ 32	31-26	< 26	≤ 1	> 4
	H. pylori			≥ 30	-	< 30	≤ 4	> 4

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NEO-SENSITABS	POTENCY	CODE	Zone diameter in mm S	I	R	Break-points MIC µg/ml S	R
Trimethoprim+Sulfa *S. pneumoniae*	5.2+240 µg	TR+SU	≥ 32	31-28	< 28	≤ 0.5/9.5	> 2/38
Vancomycin *S. pneumoniae*	5 µg	VAN.5	≥ 16	-	-	≤ 1	-
Metronidazole *H. pylori*	16 µg	MTR16	≥ 26	-	< 26	≤ 8	> 8

Remarks:

Haemophilus:

a) Beta-lactamase negative, ampicillin resistant strains (BLNAR) are best detected using Ampicillin 2.5 µg Neo-Sensitabs. BLNAR isolates must be considered resistant to amoxycillin, amoxycillin+clavulanate, as well as first and second generation cephalosporins, no matter the size of the inhibition zone.

b) Strains with reduced sensitivity to ciprofloxacin (MIC ≥ 0.125 µg/ml) show decreased sensitivity to all quinolones.

S. pneumoniae:

c) Oxacillin 1 µg is used for the detection of strains with reduced sensitivity to penicillin in pneumococci. Penicillin resistant isolates from the meninges must be considered resistant to ampicillin/amoxycillin, amoxycillin+ clavulanate and first and second generation cephalosporins.

d) Cefotaxime and ceftriaxone must not be tested against pneumococci by the diffusion method. A surrogate test is used instead: Ceftizoxime. Ceftizoxime detects reduced sensitivity to third generation cephalosporins. Strains sensitive to ceftizoxime show currently MIC < 0.5 µg/ml towards cefotaxime/ceftriaxone (susceptible), while isolates resistant to ceftizoxime should be tested by an MIC method.

e) Erythromycin. Interpretation valid for azithromycin and clarithromycin.

Gonococci:

f) Ciprofloxacin resistant gonococci should presumably be considered resistant to all quinolones.

g) A positive beta-lactamase test predicts resistance to penicillin, amoxycillin/ampicillin, piperacillin and ticarcillin.

h) Oxacillin 1 µg Neo-Sensitabs are useful to detect beta-lactamase negative gonococci with decreased sensitivity to penicillin due to chromosomal resistance.

Meningococci:

i) Oxacillin 1 µg is used routinely for the detection of reduced sensitivity to penicillins in meningococci (chromosomal resistance).

Helicobacter pylori:

For species showing slow growth it may be difficult to estabish good correlation between MIC's and zone sizes. Use an MIC method.

References:

1) Interpretation for susceptibility tests and susceptibility criteria for antibacterials in the Netherlands. CRG. Ned. Tijdschr. Med. Microbiol. 8, 79-81, 2000.

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10.3 III - Interpretation according to MIC Breakpoints of SRGA in Sweden

III-a

Rapidly growing bacteria. Interpretation according to the MIC break-points recommended by the "Swedish Reference Group for Antibiotics" (SRGA). (1)
Media: Iso-sensitest. Inoculum acc. to ICS

				Zone diameter in mm			Break-points MIC µg/ml	
	NEO-SENSITABS	POTENCY	CODE	S	I	R	S	R
	Beta-lactams:							
	Penicillin Low	5 µg	PEN.L					
	Staphylococcus spp.			≥ 28	-	< 28	≤ 0.12	Penicillinase
	Enterococcus spp.			≥ 26	25-10	< 10	≤ 0.25	> 4
	Oxacillin	1 µg	OXA.1					
j)	*S. aureus*			≥ 16	-	< 16	≤ 1	> 1
j)	Coag. neg. staph.			≥ 20	-	< 20	≤ 0.25	≥ 0.5 (CLSI)
	Cloxacillin	500 µg	CL500	Detection of plasmid mediated AmpC				
	Ampicillin	33 µg	AMP33					
a)	Enterobacteriaceae			≥ 32	31-18	< 18	≤ 1	> 8
	Enterococcus spp., *Pr. mirabilis*			≥ 26	25-18	< 18	≤ 2	> 8
d)	Amoxycillin+Clav.	30+15 µg	AM+CL	≥ 28	27-18	< 18	≤ 2+1	> 8+4
c)	Mecillinam	33 µg	MECIL	≥ 30	29-20	< 20	≤ 1	> 8
	Pr.mirabilis			≥ 24	23-20	< 20	-	-
	Piperacillin	100 µg	PIPRA	≥ 28	27-24	< 24	≤ 16	> 16
	Piperacillin+Tazobactam	100+10µg	PI+TZ	≥ 28	27-24	< 24	≤ 16	> 16
	Cephalothin	66 µg	CLOTN	≥ 34	33-20	< 20	≤ 1	> 8
	Cephalexin	30 µg	CFLEX	≥ 30	29-18	< 18	≤ 1	> 8
	Cefadroxil	30 µg	CFDRO	≥ 30	29-18	< 18	≤ 1	> 8
d)	Cefaclor	30 µg	CCLOR	≥ 30	29-18	< 18	≤ 1	> 8
	Cefuroxime (inj.)	60 µg	CEFUR	≥ 26	25-23	< 23	≤ 8	> 8
d)	Cefoxitin	**60 µg**	CFOXT	≥ 26	25-20	< 20	≤ 4	> 4
j)	*S. aureus* and *S. lugdunensis*			≥ 30	29-28	< 28	Oxa S	Mec A pos.
j)	Coag. neg. staph.			≥ 34	33-32	< 32	Oxa S	Mec A pos.
	Cefoxitin	**10 µg**	CFO10					
	S. aureus and *S. lugdunensis*			≥ 22	-	< 22	Oxa S	Mec A pos.
	Coag. neg. staph.			≥ 27	26-22	≤ 21	Oxa S	Mec A pos.
i)	Cefpodoxime	30 µg	CFPOX	≥ 28	-	< 28	≤ 1	Screen ESBL
	Cefotaxime	30 µg	CFTAX					
	Enterobacteriaceae			≥ 34	32-28	< 28	≤ 0.5	> 1
	Ceftriaxone	30 µg	CETRX					
	Enterobacteriaceae			≥ 34	33-28	< 27	≤ 0.5	> 1
	Ceftazidime	30 µg	CEZDI					
	Enterobacteriaceae			≥ 28	27-24	< 24	≤ 2	> 4
	Pseudomonas spp./*Acinetobacter* spp.			≥ 26	-	< 26	≤ 8	> 8
	Ceftazidime+Clav.	30+10 µg	CZ+CL	Detection of ESBL				
	Cefepime	30 µg	CFEPM					
	Enterobacteriaceae			≥ 34	33-28	< 28	≤ 0.5	> 1
	Pseudomonas spp./*Acinetobacter* spp.			≥ 28	22-20	< 28	≤ 8	> 8
	Cefepime+Clav.	30+10 µg	CP+CL	Detection of ESBL				
	Aztreonam	30 µg	AZTRM					
	Enterobacteriaceae			≥ 34	33-28	< 28	≤ 0.5	> 1

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	NEO-SENSITABS	POTENCY	CODE	Zone diameter in mm S	I	R	Break-points MIC µg/ml S	R
	Imipenem	15 µg	IMIPM					
g)	Enterobacteriaceae/*Acinetobacter* spp.			≥ 28	27-18	< 18	≤ 1	> 8
g)	*Pseudomonas* spp.			≥ 26	25-20	< 20	≤ 4	> 8
m)	Imipenem+EDTA	15+750µg	IM+ED	Detection of metallo-ß-lactamases				
	Meropenem	10 µg	MEROP					
	Enterobacteriaceae			≥ 34	33-16	< 16	≤ 0.12	> 8
g)	*Pseudomonas* spp.			≥ 30	29-20	< 20	≤ 2	> 8
	Acinetobacter spp.			≥ 30	29-20	< 20	≤ 1	> 8
	Ertapenem	10 µg	ETP10					
	Enterobacteriaceae			≥ 32	31-26	< 26	≤ 0.25	> 1
Aminoglycosides:								
b)	Amikacin	40 µg	AMIKA					
	Enterobacteriaceae/*Acinetobacter* spp.			≥ 24	23-20	< 20	≤ 8	> 16
	Pseudomonas spp.			≥ 26	25-22	< 22	≤ 8	> 16
	S. aureus			≥ 24	23-20	< 20	≤ 8	> 16
	Coag. neg. staph.			≥ 30	29-26	< 26	≤ 8	> 16
b) k)	Gentamicin	40 µg	GEN40					
	Enterobacteriaceae/*Acinetobacter* spp.			≥ 26	25-22	< 22	≤ 2	> 4
	Pseudomonas spp.			≥ 28	27-24	< 24	≤ 4	> 4
k)	*S. aureus*			≥ 30	29-26	< 26	≤ 1	> 1
k)	Coag. neg. staph.			≥ 34	-	< 34	≤ 1	> 1
b) k)	Netilmicin	40 µg	NETIL					
	Enterobacteriaceae/*Acinetobacter* spp.			≥ 26	25-22	< 22	≤ 2	> 4
	Pseudomonas spp.			≥ 28	27-24	< 24	≤ 4	> 4
	S. aureus			≥ 30	29-26	< 26	≤ 1	> 1
	Coag. neg. staph.			≥ 34	-	< 34	≤ 1	> 1
b)	Tobramycin	40 µg	TOBRA					
	Enterobacteriaceae/*Acinetobacter* spp.			≥ 26	25-22	< 22	≤ 2	> 4
	Pseudomonas spp.			≥ 28	27-24	< 24	≤ 4	> 4
	S. aureus			≥ 30	29-26	< 26	≤ 1	> 1
	Coag. neg. staph.			≥ 34	-	< 34	≤ 1	> 1
Others:								
	Erythromycin (staph.)	78 µg	ERYTR	≥ 30	29-26	< 26	≤ 0.5	> 0.5
	Azitromycin (staph.)	30 µg	AZITR	≥ 24	23-20	< 20	≤ 0.5	> 0.5
	Claritromycin (staph.)	30 µg	CLARI	≥ 24	23-20	< 20	≤ 0.5	> 0.5
	Clindamycin (staph.)	25 µg	CLIND	≥ 30	29-26	< 26	≤ 0.5	> 2
	Telithromycin (staph.)	15 µg	TEL15	Use Erythromycin				
e)	Fucidin	100 µg	FUCID	≥ 32	-	< 32	≤ 0.5	> 0.5
	Doxycycline	80 µg	DOXYC	≥ 28	27-23	< 23	≤ 2	> 2
	Tetracyclines	80 µg	TET80	≥ 28	27-23	< 23	≤ 2	> 2
	Chloramphenicol	60 µg	CLR60	≥ 26	-	< 26	≤ 8	> 8
	Rifampicin	30 µg	RIFAM	≥ 32	-	< 32	≤ 1	> 1
	Quinupristin/Dalfopristin	15 µg	SYN15	≥ 20	19-16	< 16	≤ 1	> 2
	Linezolid	30 µg	LINEZ	≥ 26	25-22	< 22	≤ 4	> 4
l) n)	Teicoplanin	30 µg	TPN30	≥ 16	15-14	< 14	≤ 4	> 8
p)	2+18 h. prediffusion			-	-	< 20	-	VISA/GISA
l) n)	Vancomycin	5 µg	VAN.5	≥ 16	15-14	< 14	≤ 4	> 8
p)	2+18 h. prediffusion (staph.)			-	-	< 22	-	VISA/GISA
	2+18 h. prediffusion (enterococci)			-	-	< 16	-	VRE
f)	Nitrofurantoin (U)	260 µg	NITRO	≥ 24	-	< 24	≤ 32	> 32
h)	Nalidixan (U)	130 µg	NALID	≥ 28	27-23	< 23	≤ 8	> 16
	Enterobacteriaceae			-	-	< 28	Reduced susceptibility to quinolones.	
	Norfloxacin (U)	10 µg	NORFX	≥ 28	27-24	< 24	≤ 0.5	> 1

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	NEO-SENSITABS	POTENCY	CODE	Zone diameter in mm S	I	R	Break-points MIC µg/ml S	R
	Ciprofloxacin	10 µg	CIP10					
	Enterobacteriaceae/*Acinetobacter* spp.			≥ 28	27-24	< 24	≤ 0.5/1	> 1
	Pseudomonas spp.			≥ 30	29-27	< 27	≤ 0.5	> 1
	Ofloxacin	10 µg	OFLOX	≥ 28	27-24	< 24	≤ 0.5	> 1
	Levofloxacin	5 µg	LEVOF	≥ 26	25-23	< 23	≤ 1	> 2
	Pseudomonas spp.			≥ 28	27-24	< 24	≤ 1	> 2
	Moxifloxacin	5 µg	MOXIF	≥ 28	27-24	< 24	≤ 0.5	> 1
	Staphylococcus spp.			≥ 30	29-26	< 26	≤ 0.5	> 1
p)	Colistin	10 µg	CO.10					
	2+18 h. prediffusion			≥ 15	-	< 15	≤ 2	> 2
p)	Daptomycin	30 µg	DAPCa					
	2+18 h. prediffusion							
	Staphyloccus spp			≥ 22	-	-	≤ 1	> 1
	Enterococcus spp.			≥ 12	-	-	≤ 4	-
d)	Sulphonamides (U)	240 µg	SULFA	≥ 22	-	< 22	≤ 256	> 256
	Trimethoprim	5.2 µg	TRIME	≥ 20	19-16	< 16	≤ 2	> 4
	Trimethoprim+Sulfa	5.2+240 µg	TR+SU	≥ 28	27-23	< 23	≤ 16 (1/20)	> 32
d)	Novobiocin (U)	5 µg	NOVO5	≥ 16	-	< 16	-	-
d)	Mupirocin	10 µg	MUPIR					
	Staphylococcus spp.			≥ 20	-	< 20	≤ 2	> 2
	Tigecycline	15 µg	TIG15					
	Enterobacteriaceae/enterococci			≥ 23	-	< 23	≤ 1	> 2
	Staphylococcus spp.			≥ 25	-	< 25	≤ 0.5	> 0.5

Remarks:

Staphylococci do not need to be tested against Ampicillin, Amoxycillin, and Piperacillin (use **Penicillin Low**). Staphylococci resistant to Penicillin Low (beta-lactamase producers) should be reported as resistant to the above mentioned penicillins.

a) Valid for amoxycillin. Including azidocillin with enterococci.
Klebsiella and Enterobacter are always reported **R** to ampicillin.

b) For aminoglycosides the intermediate category is only valid in case of UTI.
For other tests the intermediate results should be considered as resistant.

c) Mecillinam, when using Mueller-Hinton, use: **S:** ≥ 24 mm; **I:** 23-16 mm; **R:** < 16 mm. When testing against *P. mirabilis*, use: **S:** ≥ 20 mm, **I:** 19-16 mm, **R:** < 16 mm.

d) Break-points not yet established by the SRGA.

e) If the media contain blood, use: **S:** ≥ 28 mm; **R:** < 28 mm.

f) Klebsiella, Enterobacter and *Proteus* spp. should be reported R to nitrofurantoin.

g) Carbapenem testing in Iso-sensitest Agar may give falsely susceptibility for isolates that harbour metallo-beta-lactamases; testing on Mueller-Hinton Agar may be preferred (BSAC 2001).

h) Nalidixan is a good screening for the detection of decreased fluoroquinolone susceptibility in *Salmonella* spp. (Hakanen A. et al: J. Clin. Microbiol. **37**, 3572-77, 1999). Strains resistant to Nalidixan Neo-S (zone < 28 mm) show a decreased susceptibility to quinolones (ciprofloxacin MIC ≥ 0.125 µg/ml).

i) Strains showing zone ≤ 24 mm with Cefpodoxime Neo-Sensitabs, should be suspected of producing ESBL (*E.coli*, Klebsiella, Salmonella). For confirmatory tests use Ceftazidime+Clavulanate and Cefepime+Clavulanate compared to Ceftazidime and Cefepime Neo-Sensitabs (see ESBL).

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j) For the detection of methicillin/oxacillin resistance in staphylococci use Mueller-Hinton Agar. Iso-sensitest will not reliably detect resistance in these organisms (BSAC 2001).
Cefoxitin is very useful to detect mec A positive strains in staphylococci.

k) Staphylococci resistant to gentamicin should be reported as resistant to netilmicin and tobramycin. Gentamicin is recommended as the test drug for staphylococci, because netilmicin might give false sensitive results with coagulase negative staphylococci. Isolates sensitive to gentamicin will currently be sensitive to netilmicin.

l) Iso-sensitest cannot be used to detect vancomycin resistant enterococci (van B). Use Mueller-Hinton Agar.

m) Iso-sensitest is not recommended for detection of metallo-beta-lactamases. Use Mueller-Hinton Agar.

n) For detection of VISA, GISA, hVISA strains, see chapter in User's Guide on detection of staphylococci with decreased susceptibility to vancomycin, page 86.

o) When testing enterococci, use Gentamicin 250 µg Neo-Sensitabs to detect HLR. Strains that are HLR to gentamicin are HLR to alle aminoglycosides except streptomycin. May be tested with Streptomycin 500 µg.

p) Prediffusion technique (2+18 hours) described on page 18.

References:
1) RAF. Referens och Metodbok. Resistensbestämning av bakterier mot Antibiotika, March 1990.
2) Kompletering av RAF's Reference och Metodbok. Juni 1995.
3) RAF. Nyheter 2006.

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III-b

Haemophilus spp., *Moraxella catarrhalis*, *S. pneumoniae*, *Streptococcus* spp. and *Corynebacterium jeikeium/urealyticum*
Interpretation according to SRGA 2002
Media: Iso-sensitest + 5 % blood + NAD and 5 % CO_2
Inoculum acc. to ICS

	NEO-SENSITABS	POTENCY	CODE	Zone diameter in mm S	I	R	Break-points MIC µg/ml S	R
	Penicillin Low (G)	5 µg	PEN.L					
	Haemophilus spp.			≥ 20	19-18	< 18	≤ 1	> 1
	Streptococcus spp.			≥ 26	25-20	< 20	≤ 0.25	> 1
a)	*Moraxella catarrhalis*			BL neg	-	BL pos	Test beta-lact.	
	Penicillin Low (V)	5 µg	PEN.L					
h)	*Haemophilus* spp.			≥ 30	29-14	< 14	≤ 0.5	> 4
	Oxacillin	1 µg	OXA.1					
b)	*S. pneumoniae*			≥ 24	≤ 23	< 23	≤ 0.06 (pen)	MIC
c)	*Streptococcus* spp.			≥ 17	-	< 17	≤ 0.12 (pen)	MIC
	Ampicillin	**2.5 µg**	AMP.L					
d)	*Haemophilus* spp.			≥ 22	21-19	< 18	≤ 0.5	> 0.5
	Streptococcus spp.			≥ 24	23-18	< 18	≤ 0.25	> 1
a)	*Moraxella catarrhalis*			BL neg	-	BL pos	Test beta-lact.	
	Ampicillin	**33 µg**	AMP33					
d)	*Haemophilus* spp.			≥ 34	33-30	< 30	≤ 0.5	> 0.5
	Streptococcus spp.			≥ 36	35-28	< 28	≤ 0.25	> 1
a)	*Moraxella catarrhalis*			BL neg	-	BL pos	Test beta-lact.	
*)	Amoxycillin+Clav.	30+15 µg	AM+CL					
	Haemophilus spp.			≥ 30	29-26	< 26	≤ 2	> 4
	Cefuroxime	60 µg	CEFUR					
	Haemophilus spp.			≥ 34	33-28	< 28	≤ 2	> 2
	Moraxella catarrhalis			≥ 34	33-28	< 28	≤ 2	> 2
	Cefotaxime	30 µg	CFTAX					
	Haemophilus spp.			≥ 38	37-28	< 28	≤ 0.12	> 1
e)	*S. pneumoniae* (use ceftizoxime)							
	Ceftriaxone	30 µg	CETRX					
	Haemophilus spp.			≥ 38	37-28	< 28	≤ 0.12	> 1
e)	*S. pneumoniae* (use ceftizoxime)							
	Ceftizoxime	30 µg	CEZOX					
e)	*S. pneumoniae* (3rd gen. cephalosporins)			≥ 32	-	< 32	≤ 0.5 (Cftax, Cetrx)	MIC
	Aztreonam	30 µg	AZTRM					
	Haemophilus spp.			≥ 40	39-34	< 34	≤ 0.5	> 1
	Imipenem	15 µg	IMIPM					
	Haemophilus spp.			≥ 34	33-28	< 28	≤ 1	> 2
	S. pneumoniae			≥ 36	35-28	< 28	≤ 0.06	> 0.5
	Meropenem	10 µg	MEROP					
	Haemophilus spp.			≥ 34	33-28	< 28	≤ 1	> 2
	S. pneumoniae			≥ 36	35-26	< 26	≤ 0.06	> 0.5

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	NEO-SENSITABS	POTENCY	CODE	Zone diameter in mm S	I	R	Break-points MIC µg/ml S	R
f)	Erythromycin	78 µg	ERYTR					
j)	*S. pneumoniae*/strep.			≥ 28	-	< 28	≤ 0.5	> 0.5
	Moraxella catarrhalis			≥ 32	31-22	< 22	≤ 0.5	> 4
	C. jeikeium/urealyticum			≥ 32	31-22	< 22	≤ 0.5	> 4
f)	Clarithromycin	30 µg	CLARI					
	S. pneumoniae/strep.			test ERY				
	Moraxella catarrhalis			≥ 26	25-17	< 17	≤ 0.5	> 4
f)	Azithromycin	30 µg	AZITR					
	S. pneumoniae/strep.			test ERY				
	Moraxella catarrhalis			≥ 26	25-21	< 21	≤ 0.5	> 2
	Telithromycin	µg						
	S. pneumoniae/strep.			≥ 26	25-18	< 18	≤ 0.125	> 1
	Moraxella catarrhalis			≥ 24	23-18	< 18	≤ 0.5	> 1
	Clindamycin	25 µg	CLIND					
	S. pneumoniae/strep.			≥ 32	31-26	< 26	≤ 0.5	> 2
	Chloramphenicol	**10 µg**	CLR10					
	Haemophilus spp.			≥ 23	22-20	< 20	≤ 2	> 2
	S. pneumoniae			≥ 16	15-14	< 14	≤ 8	> 8
	Chloramphenicol	**60 µg**	CLR60					
	Haemophilus spp.			≥ 34	33-30	< 30	≤ 2	> 2
	S. pneumoniae			≥ 26	25-23	< 23	≤ 8	> 8
	Tetracyclines	80 µg	TET80					
	Haemophilus spp.			≥ 28	27-23	< 23	≤ 2	> 2
	S. pneumoniae/strep.			≥ 28	27-23	< 23	≤ 2	> 2
	Moraxella catarrhalis			≥ 28	27-23	< 23	≤ 2	> 2
	Rifampicin	30 µg	RIFAM					
	S. pneumoniae/strep.			≥ 32	31-28	< 28	≤ 1	> 1
	Linezolid	30 µg	LINEZ					
	S. pneumoniae/strep.			≥ 28	27-23	< 23	≤ 2	> 4
	Quinupristin/Dalfopristin	15 µg	SYN15					
	S. pneumoniae			≥ 20	19-17	< 17	≤ 1	> 2
	Vancomycin	5 µg	VAN.5					
	S. pneumoniae/strep.			≥ 18	17-16	< 16	≤ 4	> 4
	C. jeikeium/urealyticum			≥ 18	17-16	< 16	≤ 4	> 4
	Teicoplanin	60 µg	TEICO					
	S. pneumoniae/strep.			≥ 18	17-16	< 16	≤ 4	> 4
	C. jeikeium/urealyticum			≥ 18	17-16	< 16	≤ 4	> 4
	Fucidin	100 µg	FUCID					
	C. jeikeium/urealyticum			≥ 28	27-24	< 24	≤ 0.5	> 0.5
	Norfloxacin	10 µg	NORFX				Decreased susceptibility	
	Pneumococcus spp.			-	-	< 16	to quinolones	
	Nalidixan	130 µg	NALID				Decreased susceptibility	
	Haemophilus spp.			-	-	< 30	to quinolones	
	Ciprofloxacin	10 µg	CIP10					
i)	*Haemophilus* spp.			≥ 30	29-26	< 26	≤ 0.5	> 0.5
g)	*S. pneumoniae*			≥ 36	35-23	< 23	≤ 0.12	> 2
	Moraxella catarrhalis			≥ 30	29-26	< 26	≤ 0.5	> 0.5
	Ofloxacin	10 µg	OFLOX					
	Haemophilus spp.			≥ 30	29-26	< 26	≤ 0.5	> 0.5
g)	*S. pneumoniae*			≥ 36	35-20	< 20	≤ 0.25	> 2
	Moraxella catarrhalis			≥ 30	29-26	< 26	≤ 0.5	> 0.5
	Levofloxacin	5 µg	LEVOF					
	Haemophilus spp.			≥ 30	29-26	< 26	≤ 0.5	> 0.5
	S. pneumoniae			≥ 22	21-19	< 19	≤ 2	> 2
	Moraxella catarrhalis			≥ 30	29-26	< 26	≤ 0.25	> 0.5

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NEO-SENSITABS	POTENCY	CODE	Zone diameter in mm S	I	R	Break-points MIC µg/ml S	R
Moxifloxacin	5 µg	MOXIF					
Haemophilus spp.			≥ 30	29-26	< 26	≤ 0.5	> 0.5
S. pneumoniae			≥ 26	25-21	< 21	≤ 1	> 2
Moraxella catarrhalis			≥ 30	29-26	< 26	≤ 0.5	> 0.5
Trimethoprim+Sulfa	5.2+240 µg	TR+SU					
Haemophilus spp.			≥ 32	31-26	< 26	≤ 16	> 32
S. pneumoniae/strep.			≥ 32	31-26	< 26	≤ 16	> 32
Moraxella catarrhalis			≥ 32	31-26	< 26	≤ 16	> 32

Remarks:

a) Beta-lactamase production in *M. catarrhalis* can be detected by measuring the zones of inhibition around Amoxycillin and Amoxycillin+Clavulanate Neo-Sensitabs. If the zone around Amoxycillin+Clavulanate is > 5 mm larger than around Amocyxillin alone, the strain produces beta-lactamase (BRO-1, BRO-2). Beta-lactamase positive isolates are resistant to penicillin, amoxycillin, ampicillin, piperacillin and ticarcillin.

b) Oxacillin 1 µg is used for the detection of reduced sensitivity to penicillin in pneumococci. Penicillin-resistant isolates from the meninges must be considered resistant to ampicillin/amoxycillin, amoxycillin+clavulanate, first and second generation cephalosporins.

c) Oxacillin 1 µg may be used for the detection of viridans streptococci with reduced sensitivity to penicillin. Strains with zones ≤ 16 mm should be tested by an MIC method.

d) Beta-lactamase negative, ampicillin-resistant strains (BLNAR) are best detected using Ampicillin 2.5 µg Neo-Sensitabs. BLNAR isolates must be considered resistant to amoxycillin, amoxycillin+clavulanate as well as first and second generation cephalosporins, no matter the size of the inhibition zone. Cephalothin or cefaclor may be used as "surrogate" tablets for screening BLNAR.

e) Cefotaxime and ceftriaxone must not be tested against penumococci by the diffusion method (false results). A surrogate test, ceftizoxime, is used instead. Ceftizoxime detects reduced sensitivity to third generation cephalosporins. Strains sensitive to ceftizoxime show currently MIC < 0.5 µg/ml towards cefotaxime/ceftriaxone (susceptible), while isolates resistant to ceftizoxime should be tested by an MIC method.

f) Erythromycin is the representative of the macrolide group. The normal population of *Haemophilus* spp. is categorized as intermediate to erythromycin.

g) The break-points are chosen in such a way that the normal population of pneumococci is categorized as intermediate to ciprofloxacin and ofloxacin.

h) The break-points are chosen in such a way that the normal population of *Haemophilus* spp. is categorized as intermediate to penicillin V (oral).

i) Strains with reduced sensitivity to ciprofloxacin (MIC ≥ 0.125 µg/ml) show decreased sensitivity to all quinolones.

j) Erythromycin. Interpretation valid for azithromycin and clarithromycin.

*) Break-points not yet established by the SRGA.

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10.4 IV - Interpretations according to MIC Breakpoints of the Norwegian AFA Group

IV-a

Susceptibility testing of rapidly growing bacteria using Neo-Sensitabs and break-points recommended by the Norwegian AFA Group (January 2006 Version 1.9). Inoculum according to ICS. Media: Mueller-Hinton, Iso-Sensitest

	NEO-SENSITABS	POTENCY	CODE	Zone diameter in mm S	I	R	Break-points MIC µg/ml S	R
	Penicillins:							
	Ampicillin	33 µg	AMP33					
a)	*Enterobacteriaceae*			≥ 34	33-20	< 20	≤ 0.5	> 8
b)	*Enterococcus* spp.			≥ 26	25-18	< 18	≤ 2	> 8
	Amoxicillin	30 µg	AMOXY					
a)	*Enterobacteriaceae*			≥ 34	33-20	< 20	≤ 0.5	> 8
	Enterococcus spp.			≥ 28	27-20	< 20	≤ 2	> 8
	Amoxycillin+Clav.	30+15 µg	AM+CL					
	Enterobacteriaceae			≥ 34	33-20	< 20	≤ 0.5	> 8
	Penicillin Low	5 µg	PEN.L					
	Staphylococcus spp.			≥ 32	31-28	< 28	≤ 0.06	> 0.125 BL pos.
	Enterococcus spp.			≥ 12	-	no zone	≤ 4	> 8
	Oxacillin	1 µg	OXA.1					
c) d)	*S. aureus*			≥ 16	-	< 16	≤ 2	> 2
d) e)	*Coag. neg. staph.*			≥ 20	-	< 20	≤ 0.25	> 0.25
	Mecillinam (U)	33 µg	MECIL					
	Enterobacteriaceae			≥ 26	25-20	< 20	≤ 2	> 8
	Enterobacteriaceae M-H agar			≥ 22	21-17	< 17	≤ 2	> 8
	Piperacillin+Tazobactam	100+10µg	PI+TZ					
	Enterobacteriaceae			≥ 30	29-24	< 24	≤ 8	> 16
	Cloxacillin	500 µg	CL500	Detection of plasmid mediated Amp C				
	Cephalosporins and cephamycins:							
	Cefazolin	66 µg	CFZOL					
	Enterobacteriaceae			≥ 34	33-20	< 20	≤ 1	> 8
	Cephalexin (U)	30 µg	CFLEX					
	Enterobacteriaceae			≥ 30	29-18	< 18	≤ 1	> 8
t)	Cefuroxime	60 µg	CEFUR					
	Enterobacteriaceae			≥ 36	35-23	< 23	≤ 0.5	> 8
	Cefepime	30 µg	CFEPM	}				
	Cefepime+Clavulanate	30+10µg	CP+CL	}	Detection of ESBL			
	Cefotaxime	30 µg	CFTAX					
f)	*Enterobacteriaceae*			≥ 30	29-22	< 22	≤ 1	> 4
	Ceftriaxone	30 µg	CETRX					
f)	*Enterobacteriaceae*			≥ 30	29-22	< 22	≤ 1	> 4
	Ceftazidime	30 µg	CEZDI					
f)	*Enterobacteriaceae*			≥ 30	29-18	< 18	≤ 1	> 8
g)	*Pseudomonas*			≥ 30	29-20	< 20	≤ 2	> 8
	Ceftazidime + Clav.	30+10µg	CZ+CL		Detection of ESBL			
	Cefpodoxime	30 µg	CFPOX	-	-	< 28	≤ 1	Screen ESBL

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	NEO-SENSITABS	POTENCY	CODE	Zone diameter in mm S	I	R	Break-points MIC µg/ml S	R
	Cefpirome	30 µg	CFPIR					
	Enterobacteriaceae			≥ 30	29-18	< 18	≤ 1	> 8
g)	*Pseudomonas*			≥ 30	29-20	< 20	≤ 2	> 8
d)	Cefoxitin	60 µg	CFOXT					
	Staphylococcus aureus			≥ 30	29-28	< 28	Oxa S	mec A pos.
	Coag. neg. staph.			≥ 34	33-32	< 32	Oxa S	mec A pos.
	Cefoxitin	10 µg	CFO10					
	S. aureus (Iso-Sensitest)			≥ 22	-	< 22	Oxa S	mec A pos.
	S. aureus (Mueller-Hinton)			≥ 18	-	< 18	Oxa S	mec A pos.
	Monobactams:							
	Aztreonam	30 µg	AZTRM					
f)	*Enterobacteriaceae*			≥ 30	29-18	< 18	≤ 1	> 8
g)	*Pseudomonas*			≥ 30	29-20	< 20	≤ 2	> 8
	Carbapenems:							
	Meropenem	10 µg	MEROP					
h)	*Enterobacteriaceae*			≥ 30	29-23	< 23	≤ 0.5	> 2
g)	*Pseudomonas*			≥ 28	27-20	< 20	≤ 4	> 8
	Imipenem	15 µg	IMIPM					
h)	*Enterobacteriaceae*			≥ 32	31-24	< 24	≤ 0.5	> 2
g)	*Pseudomonas,Enterococcus faecalis*			≥ 26	25-20	< 20	≤ 4	> 8
	Imipenem+EDTA	15+750 µg	IMIED	Detection of metallo-ß-lactamases			≤ 4	> 8
y)	Ertapenem	10 µg	ETP10					
	Enterobacteriaceae			≥ 30	-	< 30	≤ 0.25	> 2
	Glycopeptides:							
	Vancomycin	5 µg	VAN.5	≥ 16	15-14	< 14	≤ 4	> 8
i) x) z)	2+18 h. prediffusion (staph.)			-	-	< 22	-	VISA/GISA
	2+18 h. prediffusion (enterococci)			-	-	< 16	-	VRE
	Teicoplanin	30 µg	TPN30	≥ 16	15-14	< 14	≤ 4	> 8
i) x) z)	2+18 h. prediffusion			-	-	< 20	-	VISA/GISA
y)	Daptomycin	30 µg	DAPCa					
z)	2+18 h. prediffusion							
	Staphylococcus spp.			≥ 22	-	-	≤ 1	-
	Enterococcus spp.			≥ 12	-	-	≤ 4	-
	Macrolides:							
j)	Erythromycin	78 µg	ERYTR					
u)	*Staphylococcus* spp., *Enterococcus* spp.			≥ 28	27-24	< 24	≤ 1	> 2
	Clarithromycin	30 µg	CLARI					
u)	*Staphylococcus* spp., *Enterococcus* spp.			≥ 22	21-18	< 18	≤ 1	> 2
	Azithromycin	30 µg	AZITR					
u)	*Staphylococcus* spp., *Enterococcus* spp.			≥ 22	21-18	< 18	≤ 1	> 2
	Lincosamides:							
	Clindamycin	25 µg	CLIND					
u)	*Staphylococcus* spp.			≥ 26	25-22	< 22	≤ 1	> 2
	Aminoglycosides:							
	Gentamicin	**40 µg**	GEN40					
	Enterobacteriaceae			≥ 26	25-22	< 22	≤ 2	> 4
k)	*Staphylococcus* spp.			≥ 30	29-26	< 26	≤ 1	> 1

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	NEO-SENSITABS	POTENCY	CODE	Zone diameter in mm S	I	R	Break-points MIC µg/ml S	R
	Netilmicin	40 µg	NETIL					
	Enterobacteriaceae			≥ 26	25-22	< 22	≤ 2	> 4
l)	*Staphylococcus* spp.			≥ 30	29-26	< 26	≤ 1	> 1
	Tobramycin	40 µg	TOBRA					
	Enterobacteriaceae			≥ 26	25-22	< 22	≤ 2	> 4
m)	*Staphylococcus* spp.			≥ 30	29-26	< 26	≤ 1	> 1
y)	Daptomycin	30 µg	DAPCa					
z)	2+18 h. prediffusion							
	Staphylococcus spp.			≥ 22	-	-	≤ 1	-
	Enterococcus spp.			≥ 12	-	-	≤ 4	-
	Streptomycin	500 µg	ST500					
	Enterococcus spp. (HLR)			≥ 18	-	< 18	≤ 256	> 256
	Gentamicin	**250 µg**	GN500					
n)	*Enterococcus* spp. (HLR)			≥ 18	-	< 18	≤ 128	> 128
	Chloramfenicol:							
	Chloramphenicol	60 µg	CLR60	≥ 26	-	< 26	≤ 8	> 8
	Enterococcus spp.			≥ 28	-	< 28	≤ 4	> 4
	Tetracyclines:							
	Doxycycline	80 µg	DOXYC					
	Enterobacteriaceae			≥ 30	29-18	< 18	≤ 1	> 8
	Staphylococcus spp., *Enterococcus* spp.			≥ 28	27-24	< 24	≤ 1	> 2
	Tetracyclines	80 µg	TET80					
	Enterobacteriaceae			≥ 24	23-18	< 18	≤ 4	> 8
	Staphylococcus spp., *Enterococcus* spp.			≥ 28	27-24	< 24	≤ 1	> 2
	Quinolones:							
o)	Nalidixan (U)	130 µg	NALID	≥ 24	-	< 24	≤ 16	> 16
	Enterobacteriaceae			-	-	< 28	Reduced susceptibility to quinolones	
	Norfloxacin (U)	10 µg	NORFX					
	Enterobacteriaceae			≥ 28	27-23	< 23	≤ 0.5	> 1
	Ciprofloxacin	10 µg	CIP10					
o)	*Enterobacteriaceae*			≥ 28	27-23	< 23	≤ 0.5	> 1
p)	*Staphylococcus* spp.			≥ 26	25-22	< 22	≤ 1	> 1
	Enterococcus spp.			≥ 32	31-18	< 18	≤ 0.125	> 2
g)	*Pseudomonas*			≥ 28	27-23	< 23	≤ 1	> 1
	Ofloxacin	10 µg	OFLOX					
o)	*Enterobacteriaceae*			≥ 28	27-23	< 23	≤ 0.5	> 1
	Enterococcus spp.			≥ 30	29-16	< 16	≤ 0.25	> 4
q)	*Staphylococcus* spp. (U)			≥ 26	25-22	< 22	≤ 1	> 1
	Others:							
	Nitrofurantoin (U)	260 µg	NITRO	≥ 24	-	< 24	≤ 32	> 32
	Sulfonamides (U)	240 µg	SULFA					
	Enterobacteriaceae, Staphylococcus spp.			≥ 26	25-20	< 20	≤ 64	> 128
	Trimethoprim (U)	5.2 µg	TRIME	≥ 20	19-16	< 16	≤ 2	> 4
	Trimethoprim+ Sulfa	5.2+240 µg	TR+SU	≥ 28	27-23	< 23	≤ 2/38	> 8/152
	Colistin (Polymyxins)	150 µg	CO150	≥ 20	-	< 20	-	-
y)	Colistin	10 µg	CO.10					
z)	2+18 h. prediffusion			≥ 15	-	< 15	≤ 2	> 2
y)	Tigecycline	15 µg	TIG15					
	Enterobacteriaceae			≥ 23	-	< 23	≤ 1	> 2
	Staphylococcus spp.			≥ 25	-	< 25	≤ 0.5	> 0.5

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NEO-SENSITABS	POTENCY	CODE	Zone diameter in mm S	I	R	Break-points MIC µg/ml S	R
Fucidin	100 µg	FUCID					
r) *Staphylococcus* spp.			≥ 32	-	≤ 32	≤ 0.5	> 0.5
Rifampicin	30 µg	RIFAM					
Staphylococcus spp., *Enterococcus* spp.			≥ 32	-	< 32	≤ 1	> 1
Linezolid	30 µg	LINEZ					
Staphylococcus spp., *Enterococcus* spp.			≥ 26	25-22	< 22	≤ 4	> 4
Quinupristin-Dalfopristin	15 µg	SYN15					
s) *Staphylococcus* spp., *Enterococcus* spp.			≥ 18	17-16	< 16	≤ 2	> 2

(U) urine

Remarks:

a) With these MIC breakpoints, the normal population of *E. coli* is categorized as intermediate to ampicillin/ amoxycillin.
Klebsiella spp. should always be reported as resistant to ampicillin, no matter the size of the inhibition zone.

b) Reduced sensitivity to ampicillin is common in *E. faecium*. The MIC distribution of *E. faecalis* without resistance mechanisms (normal distribution) against ampicillin is 0.125 to 2 µg/ml.

c) Staphylococci should be tested (beta-lactams) against penicillin and oxacillin only. Oxacillin-resistant staphylococci should be considered resistant to all available beta-lactam antibiotics. Iso-sensitest agar should not be used for testing staphylococci against oxacillin because of false sensitivity results (BSAC 2002).

d) Cefoxitin may be used for the detection of mecA positive staphylococci (oxacillin R). *S. aureus* resistant to cefoxitin 60 µg (zone 27 mm or less) are mecA positive.

e) Valid for *S. epidermidis*, *S. hominis* and *S. haemolyticus*. Other coagulase negative staphylococci (*S. saprophyticus*, *S. lugdunensis*, *S. xylosus*) should be tested using the zones recommended for *S. aureus* (16/14) and / or using cefoxitin.

f) Isolates being I / R (MIC > 1 µg/ml) should be tested for ESBL production (See Neo-Sensitabs User's Guide chapter 16.1).

g) The MIC breakpoints used are tentative. The AFA-group has not yet established MIC breakpoints for pseudomonas.

h) Isolates with MIC > 0.5 µg/ml might produce metallo-beta-lactamases (See User's Guide 2003, Imipenem + EDTA) or other broad spectrum beta-lactamases.
Carbapenem testing on Iso-sensitest agar may give false sensitive results with isolates able to produce metallo-beta-lactamases. Testing on Mueller-Hinton agar should be preferred (BSAC 2002).

i) Concerning detection of VISA / GISA strains and the detection of vancomycin resistant enterococci (VRE) see Neo-Sensitabs User's Guide chapter 14.2.

j) Erythromycin is the group representative for the macrolides and results are valid for clarithromycin and azithromycin.

k) Staphylococci and enterococci resistant to gentamicin should be reported as resistant to netilmicin and tobramycin.

l) Gentamicin is recommended as test drug for staphylococci because netilmicin might give false sensitive results with coagulase negative staphylococci. Isolates sensitive to gentamicin will currently be sensitive to netilmicin.

m) Tobramycin sensitive strains will currently be sensitive to gentamicin and netilmicin.

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n) Gentamicin HLR in enterococci indicates resistance to all aminoglycosides, except streptomycin. Gentamicin sensitive *E. faecalis* (but not *E. faecium*) are also sensitive to netilmicin. ***E. faecium*** shows intrinsic resistance towards kanamycin, tobramycin and netilmicin due to the production of the enzyme AAC (6^1).

o) Isolates with MICs > 0.125 µg/ml (cipro) or MIC > 0.5 µg/ml (oflox) show reduced sensitivity to ciprofloxacin/ ofloxacin. There is a risk of resistance development during treatment. Nalidixan resistance can be used to detect strains with reduced susceptibility to the fluoquinolones.

p) The suggested breakpoint classified the normal population as intermediate.

q) Staphylococci may develop resistance during quinolone therapy. There is cross-resistance between quinolones against staphylococci.

r) Valid for systemic therapy.

s) *E. faecalis* is resistant to Quinupristin/Dalfopristin.

t) With these MIC breakpoints, the normal population of *E. coli* is categorized as intermediate to cefuroxime.

u) For detection of inducible MLS_B resistance, we refer to Neo-Sensitabs User's Guide.

x) For detection of VISA, GISA, hVISA strains, see chapter in User's Guide on detection of staphylococci with decreased susceptibility to vancomycin, page 86.

y) MIC breakpoints taken from SRGA or EUCAST.

z) Prediffusion technique (2+18 hours) is described on page 18.

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IV-b

Susceptibility testing of *Haemophilus* spp., *Streptococcus pneumoniae, Streptococcus* spp., and *Moraxella catarrhalis* using Neo-Sensitabs and breakpoints recommended by the Norwegian AFA Group (January 2006 Version 1.9) Inoculum according to ICS. Media: HTM*. Besides PDM, MH and ISO (all + 5% blood) and incubation 5-7 % CO_2**

	NEO-SENSITABS	POTENCY	CODE	Zone diameter in mm S	I	R	Break-points MIC µg/ml S	R
Penicillins:								
	Penicillin Low (G)	5 µg	PEN.L					
	Haemophilus spp.			≥ 20	19-14	< 14	≤ 1	> 4
j)	*Beta haem. streptococcus*			≥ 25	-	-	≤ 0.12	-
j)	*Viridans streptococcus*			≥ 28	27-17	< 17	≤ 0.12	> 2
a)	*Moraxella catarrhalis*			BL neg.		BL pos.	Test betalactamase prod.	
	Penicillin Low (V)	5 µg	PEN.L					
	Haemophilus spp.			≥ 30	29-14	< 14	≤ 0.5	> 4
	Oxacillin	1 µg	OXA.1					
b)	*S. pneumoniae*			≥ 24	-	< 24	≤ 0.06 (pen)	-
j)	*Streptococcus* spp.			≥ 17	-	< 17	≤ 0.12 (pen)	-
	Ampicillin	**2.5 µg**	AMP.L					
c)	*Haemophilus* spp.			≥ 20	19-16	< 16	≤ 1	> 4
	Ampicillin	**33 µg**	AMP33					
	Haemophilus spp.			≥ 32	31-26	< 26	≤ 1	> 4
d)	*S. pneumoniae (non meningeal)*			≥ 36	35-29	< 29	≤ 0.5	> 2
	Beta haem. streptococcus			≥ 30	-	-	≤ 0.25	-
	Viridans streptococcus			≥ 36	35-24	< 24	≤ 0.25	> 4
a)	*Moraxella catarrhalis*			BL neg.		BL pos.	Test betalactamase prod.	
	Amoxycillin+Clav.	30+15 µg	AM+CL					
	Haemophilus spp.			≥ 30	29-26	< 26	≤ 2	> 4
	Moraxella catarrhalis			≥ 34	33-26	< 26	≤ 1	> 4
Cephalosporins:								
	Cefuroxime	60 µg	CEFUR					
	Haemophilus spp.			≥ 34	33-28	< 28	≤ 2	> 4
	Moraxella catarrhalis			≥ 34	-	< 34	≤ 2	> 2
d)	*S. pneumoniae (non meningeal)*			≥ 38	37-32	≤ 32	≤ 0.5	> 1
	Cefotaxime	30 µg	CFTAX					
e)	*Haemophilus* spp.			≥ 38	37-28	< 28	≤ 0.125	> 1
	Moraxella catarrhalis			≥ 33	32-29	< 29	≤ 2	> 4
	Ceftizoxime	30 µg	CEZOX					
f)	*S. pneumoniae* (3rd gen cephalosporins)			≥ 32	-	< 32	≤ 0.5 (CFTAX,CETRX)	MIC
	Ceftriaxone	30 µg	CETRX					
e)	*Haemophilus* spp.			≥ 38	37-28	≤ 28	≤ 0.125	> 1
Monobactams:								
	Aztreonam	30 µg	AZTRM					
	Haemophilus spp.			≥ 40	39-32	< 32	≤ 0.5	> 2
	Moraxella catarrhalis			≥ 28	-	< 28	≤ 4	> 4

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	NEO-SENSITABS	POTENCY	CODE	Zone diameter in mm S	I	R	Break-points MIC µg/ml S	R
Carbapenems:								
	Imipenem	15 µg	IMIPM					
	Haemophilus spp.			≥ 34	-	-	≤ 1	-
	S. pneumoniae, streptococci			≥ 26	25-24	< 24	≤ 2	> 2
	Anaerobes			≥ 26	25-20	< 20	≤ 2	> 8
	Meropenem	10 µg	MEROP					
g)	*Haemophilus* spp.			≥ 36	-	-	≤ 0.5	-
	S. pneumoniae, streptococci			≥ 30	29-24	< 24	≤ 0.5	> 2
	S. pneumoniae (meningit)			≥ 32	31-24	< 24	≤ 0.25	> 2
	Anaerobes			≥ 26	25-20	< 20	≤ 2	> 8
Glycopeptides:								
	Vancomycin	5 µg	VAN.5					
	S. pneumoniae, streptococci			≥ 18	17-16	< 16	≤ 4	> 4
	Teicoplanin	30 µg	TPN30					
	S. pneumoniae, streptococci			≥ 18	17-14	< 14	≤ 4	> 4
Macrolides:								
h)	Erythromycin	78 µg	ERYTR					
	Haemophilus spp.			≥ 34	33-19	< 19	≤ 1	> 8
j)	*S. pneumoniae*, streptococci			≥ 28	-	< 28	≤ 0.5	> 0.5
	Moraxella catarrhalis			≥ 32	-	< 32	≤ 1	> 1
Lincosamides:								
	Clindamycin	25 µg	CLIND					
j)	*S. pneumoniae*, streptococci			≥ 36	35-26	< 26	≤ 0.25	> 2
Chloramphenicol:								
	Chloramphenicol	60 µg	CLR60					
	Haemophilus spp., *Moraxella catarrhalis*			≥ 34	33-30	< 30	≤ 2	> 4
	S. pneumoniae, streptococci			≥ 28	27-23	< 23	≤ 4	> 4
	Chloramphenicol	**10 µg**	CLR10					
	Haemophilus spp., *Moraxella catarrhalis*			≥ 23	22-20	< 20	≤ 2	> 4
Tetracyclines:								
	Tetracyclines	80 µg	TET80	≥ 28	27-24	< 24	≤ 2	> 2
	Tetracyclines	**10 µg**	TET10	≥ 20	19-17	< 17	≤ 2	> 2
	Doxycycline	80 µg	DOXYC					
	Haemophilus spp. HTM			≥ 28	27-24	< 24	≤ 2	> 2
	Haemophilus spp. MH/PDM			≥ 25	24-20	< 20	≤ 4	> 4
	S. pneumoniae, *Moraxella catarrhalis*			≥ 26	25-23	< 23	≤ 1	> 2
Quinolones:								
	Nalidixan	130 µg	NALID				Reduced susceptibility	
	Haemophilus spp.			-	-	< 30	to quinolones.	
	Ciprofloxacin	10 µg	CIP10					
	Haemophilus spp,			≥ 30	29-26	< 26	≤ 0.5	> 0.5
i)	*S. pneumoniae*			≥ 36	35-22	< 22	≤ 0.125	> 2
	Moraxella catarrhalis			≥ 32	31-28	< 28	≤ 0.125	> 0.5
	Norfloxacin	10 µg	NORFX				Reduced susceptibility	
	S. pneumoniae			-	-	< 16	to quinolones.	

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NEO-SENSITABS	POTENCY	CODE	Zone diameter in mm S	I	R	Break-points MIC µg/ml S	R
Others:							
Trimethoprim+Sulfa	5.2+240 µg	TR+SU	≥ 32	31-26	< 26	≤ 0.5/9.5	> 2/38
Moraxella catarrhalis			≥ 30	29-26	< 26	≤ 1/19	> 2/38
Linezolid	30 µg	LINEZ					
S. pneumoniae			≥ 28	27-23	< 23	≤ 4	> 4
Quinupristin/Dalfopristin	15 µg	SYN15					
S. pneumoniae, streptococci			≥ 18	-	< 18	≤ 2	> 2

BL pos. = Beta-lactamase producing strains, BL neg. = Non-beta-lactamase producing strains
* *Haemophilus* spp. ** *Haemophilus* spp. and *S. pneumoniae*

Remarks:

a) Beta-lactamase production in *M. catarrhalis* can be detected by measuring the zones of inhibition around Amoxycillin and Amoxycillin + Clavulanate Neo-Sensitabs. If the zone around Amoxycillin + Clavulanate is > 5 mm larger than around Amoxycillin alone, the strain produces beta-lactamase (BRO-1, BRO-2). Beta-lactamase positive isolates are resistant to penicillin, amoxycillin, ampicillin, piperacillin and ticarcillin.

b) Oxacillin 1 µg is used for the detection of reduced sensitivity to penicillin in pneumococci. Penicillin-resistant isolates from the meninges must be considered resistant to ampicillin/amoxycillin, amoxycillin + clavulanate, first and second generation cephalosporins.

c) Beta-lactamase negative, ampicillin-resistant strains (BLNAR) are best detected using Ampicillin 2.5 µg Neo-Sensitabs. BLNAR isolates must be considered resistant to amoxycillin, amoxycillin + clavulanate as well as first and second generation cephalosporins, no matter the size of the inhibition zone.

d) To be used with non-meningeal isolates only.

e) Breakpoint chosen for therapy of meningitis.

f) Cefotaxime and ceftriaxone must not be tested against pneumococci by the diffusion method (false results). A surrogate test, ceftizoxime, is used instead. Ceftizoxime detects reduced sensitivity to third generation cephalosporins. Strains sensitive to ceftizoxime show currently MIC < 0.5 µg/ml towards cefotaxime/ceftriaxone (susceptible), while isolates resistant to ceftizoxime should be tested by an MIC method.

g) Breakpoint chosen for therapy of meningitis.

h) Erythromycin is the representant of the macrolide group. The normal population of *Haemophilus* spp. is categorized as intermediate to erythromycin.

i) The breakpoints are chosen in such a way that the normal population of pneumococci is categorized as intermediate to ciprofloxacin/ofloxacin.

j) For detection of inducible MLS_B resistance, we refer to Neo-Sensitabs User's Guide.

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10.5 V - Danish Blood Agar. Interpretation Valid for Denmark

Va

Danish blood agar. Interpretation valid for Denmark
Aflæsningsskema for Neo-Sensitabs (hurtigvoksende bakterier)
DIREKTE METODE – Resistensplade (DBA)
Semikonfluerende vækst

3	≥ 30 mm
2	21-29 mm
0	≤ 20 mm

Ciprofloxacin 10 µg		i)
Gatifloxacin		i)
Imipenem (Pseud. 30/23)		
Levofloxacin		i)
Moxifloxacin		i)
Ofloxacin		i)
Spiramycin (30/26)		
Ertapenem (30/26)		r)

3	≥ 28 mm
2	23-27 mm
1	15-22 mm
0	≤ 14 mm

Ampicillin 33 µg	a)	l)
Ampicillin 33 µg	a)	l)
Cefoxitin 60 µg	b)	k)
Cefuroxime		b)
Cephalosporins		b)
Chloramphenicol 60 µg		
Clindamycin 25 µg		
Erythromycin		
Kanamycin 100 µg		
Lincomycin		
Linezolid		
Methicillin		g)
Nalidixan		n)
Neomycin		
Nitrofurantoin		h)
Penicillin Low		i)
Rifampicin		
Streptomycin 100 µg		d)
Sulphonamides		
Tetracyclines 80 µg		j)
Trimethoprim+Sulfa		

3	≥ 28 mm
2 *)	21-27 mm
1	10-20 mm
0	≤ 9 mm

Piperacillin	a)	b)
Piperacillin+Tazo.		b)
Ticarcillin	a)	b)
Ticarcillin+Clav.		b)

*) *høj dosering 1-2 g/time*

Følsom	**3**
Moderat følsom	**2**
Relativ resistent	**1**
Resistent	**0**

3	≥ 28 mm
2	20-27 mm
1	15-19 mm
0	≤ 14 mm

Amoxycillin		a)
Amoxycillin+Clav.		b)
Aztreonam		c)
Cefepime	b)	c)
Cefotaxime	b)	c)
Cefpirome	b)	c)
Cefsulodin		
Ceftazidime	b)	c)
Mecillinam	b)	p)

3	≥ 26 mm
2	23-25 mm
1	15-22 mm
0	≤ 14 mm

Amikacin		
Gentamicin 40 µg	d)	m)
Netilmicin		m)
Tobramycin		m)

3	≥ 26 mm
2	18-25 mm
1	13-17 mm
0	≤ 12 mm

Ceftriaxone	b)	c)
Imipenem		b)
Norfloxacin		i)
Meropenem (Pseud. 26/20)		

3	≥ 24 mm
2	18-23 mm
1	13-17 mm
0	≤ 12 mm

Tigecycline (25/24)		
Cefpodoxime 30 µg		o)
Cefotetan		b)
Fucidin (24/22)		

3	≥ 22 mm
2	19-21 mm
1	13-18 mm
0	≤ 12 mm

Vancomycin 5 µg		
2+18 t. prædiff. (22/21) staph.		s)
Azithromycin		
Bacitracin		
Clarithromycin		
Polymyxins 150 µg		
Telithromycin (22/16)		
Trimethoprim		
Cefoxitin 10 µg		
(*S. aureus* 22/22)		
Daptomycin		
2+18 t. prædiff. staph. (22/21)		s)

3	≥ 20 mm
2	17-19 mm
0	≤ 16 mm

Teicoplanin 30 µg		
Meropenem		b)
Quinupristin/Dalfopristin		
Teicoplanin 30 µg		
2+18 t. prædiff. (20/19)		s)

S:	≥ 20 mm
R:	≤ 19 mm

Mupirocin
Cefpodoxime 10 µg (< 20): ESBL

3	≥ 18 mm
2	16-17 mm
1	13-15 mm
0	≤ 12 mm

Fosfomycin

S:	≥ 16 mm
R:	≤ 15 mm

Novobiocin 5 µg		
Oxacillin 1 µg	f)	g)
Vancomycin 5 µg	q)	e)
2+18 t. prædiff.(enterococci)		
Colistin 10 µg		
2+18 t. prædiff. (15/14)		s)
Daptomycin		
2+18 t. prædiffusion, enterococci (12/11)		s)

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Vb

Danish blood agar. Interpretation valid for Denmark
Aflæsningsskema for Neo-Sensitabs (hurtigvoksende bakterier)
DIREKTE METODE – Resistensplade (DBA)
Semikonfluerende vækst

		NEO-SENSITABS	STYRKE	KODE	Zone diameter i mm 3	2	1	0	MIC µg/ml 3	2	1	0
a)		Amoxycillin	30 µg	AMOXY	≥ 28	20-27	15-19	≤ 14	≤ 2	3-6	8-32	> 32
b)		Amoxycillin+ Clavulanate	30+15 µg	AM+CL	≥ 28	20-27	15-19	≤ 14	≤ 2	3-6	8-32	> 32
		Amikacin	40 µg	AMIKA	≥ 26	23-25	15-22	≤ 14	≤ 4	6-8	12-64	> 64
a)	l)	Ampicillin	33 µg	AMP33	≥ 28	23-27	15-22	≤ 14	≤ 2	3-6	8-32	> 32
		Azithromycin	30 µg	AZITR	≥ 22	19-21	13-18	≤ 12	≤ 1.5	2-4	6-16	> 16
c)		Aztreonam	30 µg	AZTRM	≥ 28	20-27	15-19	≤ 14	≤ 2	3-8	16-32	> 32
j)		Chloramphenicol	60 µg	CLR60	≥ 28	23-27	15-22	≤ 14	≤ 6	8-16	32-64	> 64
b)	c)	Cefepime	30 µg	CFEPM	≥ 28	20-27	15-19	≤ 14	≤ 2	3-8	16-32	> 32
		Cefepime+Clav.	30+10µg	CP+CL	Påvisning af ESBL							
b)	c)	Cefotaxime	30 µg	CFTAX	≥ 28	20-27	15-19	≤ 14	≤ 2	3-8	16-31	> 32
b)	k)	Cefoxitin	**60 µg**	CFOXT	≥ 28	23-27	15-22	≤ 14	≤ 3-4	6-8	12-64	> 64
		S. aureus og *S. lugdunensis*			≥ 30	-	-	≤ 27	Oxa S	-	-	Mec A pos.
		Coag. neg. staph.			≥ 34	-	-	≤ 31	Oxa S	-	-	Mec A pos.
b)	k)	Cefoxitin	**10 µg**	CFO10								
		S. aureus og *S. lugdunensis*			≥ 22	-	-	< 22	Oxa S	-	-	Mec A pos.
		Coag. neg. staph.			≥ 27	22-26	-	≤ 21	Oxa S	-	-	Mec A pos.
b)	c)	Cefpirome	30 µg	CFPIR	≥ 28	20-27	15-19	≤ 14	≤ 2	3-8	16-32	> 32
o)		Cefpodoxime	**30 µg**	CFPOX	≥ 24	-	-	≤ 23	≤ 4	-	-	≥ 8
		Cefpodoxime	**10 µg**	CPD10	-	-	-	< 20	-	-	-	Screen ESBL
		Cefsulodin	30 µg	CFSUL	≥ 28	20-27	15-19	≤ 14	≤ 2	3-8	12-32	> 32
b)	c)	Ceftazidime	30 µg	CEZDI	≥ 28	20-27	15-19	≤ 14	≤ 2	3-8	12-32	> 32
		Ceftazidime+Clav.	30+10µg	CZ+CL	Påvisning af ESBL							
b)	c)	Ceftriaxone	30 µg	CETRX	≥ 26	18-25	13-17	≤ 12	≤ 2	3-8	12-32	> 32
b)		Cefuroxime	60 µg	CEFUR	≥ 28	23-27	15-22	≤ 14	≤ 3-4	6-8	12-64	> 64
b)		Cephalothin	60 µg	CLOTN	≥ 28	23-27	15-22	≤ 14	≤ 3-4	6-8	12-64	> 64
i)		Ciprofloxacin	10 µg	CIP10	≥ 30	21-29	-	≤ 20	≤ 0,25	0,5-2	-	> 2
		Clarithromycin	30 µg	CLARI	≥ 22	19-21	13-18	≤ 12	≤1,5	2-4	6-16	> 16
		Clindamycin	25 µg	CLIND	≥ 28	23-27	15-22	≤ 14	≤ 1	1,5-2	4-8	> 8
		Cloxacillin	500 µg	CL500	Påvisning af plasmid Amp C							
		Colistin	10 µg	CO.10								
s)		2+18 t prædiffusion			≥ 15	-	-	< 15	≤ 2	-	-	> 2
		Daptomycin	30 µg	DAPCa								
s)		2+18 t prædiffusion										
		Staphylococcus spp.			≥ 22	-	-	-	≤ 1	-	-	-
		Enterococcus spp.			≥ 12	-	-	-	≤ 4	-	-	-
		Erythromycin	78 µg	ERYTR	≥ 28	23-27	15-22	≤ 14	≤ 1	2-4	6-32	> 32
r)		Ertapenem	10 µg	ETP10	≥ 30	29-26	-	< 26	≤ 0.5	-	-	> 1
		Fosfomycin	70+40 µg	FOSFO	≥ 18	16-17	13-15	≤ 12	-	-	-	-
		Fucidin (3)	100 µg	FUCID	≥ 24	23	-	≤ 22	≤ 0,5	1	-	≥ 2

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	NEO-SENSITABS	STYRKE	KODE	Zone diameter i mm 3	2	1	0	MIC µg/ml 3	2	1	0
i)	Gatifloxacin	5 µg	GATIF	≥ 30	21-29	-	≤ 20	≤ 0,25	0,5-2	-	> 2
m)	Gentamicin	40 µg	GEN40	≥ 26	23-25	15-22	≤ 14	≤ 4	6-8	12-64	> 64
d)	*Enterococcus* spp.			-	-	-	< 20	-	-	-	> 500 (HLR)
b)	Imipenem	15 µg	IMIPM	≥ 26	18-25	13-17	≤ 12	≤ 2	3-8	12-16	> 16
	Pseudomonas spp.			≥ 30	24-29	-	≤ 23	≤ 2	4-8	-	> 8
	Imipenem+EDTA	15+750µg	IM+ED	Påvisning af metallo-ß-lactamase							
	Kanamycin	100 µg	KANAM	≥ 28	23-27	15-22	≤ 14	≤ 4	6-16	32-64	> 64
i)	Levofloxacin	5 µg	LEVOF	≥ 30	21-29	-	≤ 20	≤ 0,25	0,5-2	-	> 2
	Linezolid	30 µg	LINEZ	≥ 28	23-27	-	≤ 22	≤ 2	4	-	> 8
b) p)	Mecillinam (U)	33 µg	MECIL	≥ 28	20-27	15-19	≤ 14	≤ 2	3-16	32-64	> 64
g)	Methicillin	29 µg	METHI	≥ 28	23-27	15-22	≤ 14	≤ 1,5	2-6	8-32	> 32
b)	Meropenem	10 µg	MEROP	≥ 20	17-19	-	≤ 16	≤ 4	8-32	-	> 32
	Pseudomonas spp.			≥ 26	21-25	-	≤ 20	≤ 2	4-8	-	> 8
i)	Moxifloxacin	5 µg	MOXIF	≥ 30	21-29	-	≤ 20	≤ 0.25	0.5-2	-	> 2
	Mupirocin	10 µg	MUPIR	≥ 20	-	-	≤ 19	≤ 1	-	-	> 1
n)	Nalidixan (U)	130 µg	NALID	≥ 28	23-27	15-22	≤ 14	≤ 4	6-16	32-64	> 64
	Enterobacteriaceae			-	-	-	< 26	Nedsat følsomhed for Kinoloner			
	Haemophilus spp.			-	-	-	< 28				
	Gonococcer			-	-	-	< 32				
	Neomycin	120 µg	NEOMY	≥ 28	23-27	15-22	≤ 14	-	-	-	-
m)	Netilmicin	40 µg	NETIL	≥ 26	23-25	15-22	≤ 14	≤ 4	6-8	12-64	> 64
h)	Nitrofurantoin (U)	260 µg	NITRO	≥ 28	23-27	15-22	< 14	< 32	32-64	64-128	> 128
i)	Norfloxacin (U)	10 µg	NORFX	≥ 26	18-25	13-17	≤ 12	≤ 0,5	1-4	6-16	> 16
	S. pneumoniae			-	-	-	< 16	Nedsat følsomhed for Kinoloner			
	Novobiocin	5 µg	NOVO5	≥ 16	-	-	≤ 15	≤ 2	-	-	> 2
c)	Ofloxacin	10 µg	OFLOX	≥ 30	21-29	-	≤ 20	≤ 0,25	0,5-2	-	> 2
	Oxacillin	1 µg	OXA.1								
g)	*S. aureus*			≥ 16	-	-	≤ 15	≤ 1	-	-	> 1
g)	Koag. neg. staph.			≥ 20	-	-	≤ 19	≤ 0,25	-	-	> 0,5
f)	*S. pneumoniae*			≥ 24	-	-	≤ 23	≤ 0,06 (pen)	-	-	≥ 0,12
f)	*N. gonorrhoeae* (screening)			≥ 12	-	-	≤ 9	≤ 0,06 (pen)	-	-	-
	Penicillin Low	5 µg	PEN.L	≥ 28	23-27	15-22	≤ 14	≤ 0,25	0,5	1-2	> 2
	Staphylococcus spp.			≥ 28	-	-	≤ 27	≤ 0,25	-	-	BL pos.
a) b)	Piperacillin	100 µg	PIPEM	≥ 28	21-27	10-20	≤ 9	≤ 16	32-128	256	> 256
b)	Piperacillin+ Tazobactam	100+10µg	PI+TZ	≥ 28	21-27	10-20	≤ 9	≤ 16	32-128	256	> 256
	Polymyxins	150 µg	CO150	≥ 22	19-21	13-18	≤ 12	≤ 4	8-16	32-128	> 128
	Quinupristin/ Dalfopristin	15 µg	SYN15	≥ 20	19-17	-	≤ 16	≤ 1	2-4	-	> 4
	Rifampicin	30 µg	RIFAM	≥ 28	23-27	15-22	≤ 14	≤ 0,5	1-2	4-16	> 16
	Spiramycin	200 µg	SPIRA	≥ 30	27-29	-	≤ 26	≤ 2	4-8	-	> 8
j)	Streptomycin	100 µg	ST100	> 28	23-27	15-22	≤ 14	≤ 8	12-32	64-128	> 128
d)	*Enterococcus* spp.			-	-	-	< 20	-	-	-	> 1000 (HLR)
	Sulphonamides (U)	240 µg	SULFA	≥ 28	23-27	15-22	≤ 14	≤ 64	128-256	512	> 512

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	NEO-SENSITABS	STYRKE	KODE	Zone diameter i mm 3	2	1	0	MIC µg/ml 3	2	1	0
	Teicoplanin	30 µg	TPN30	≥ 20	19-17	-	≤ 16	≤ 2	4-8	-	> 16
s)	2 +18 t. prædiffusion			-	-	-	< 20	-	-	-	GISA/VISA
	Telithromycin	15 µg	TEL15	≥ 22	21-17	-	≤ 16	≤ 1	2	-	> 2
j)	Tetracyclines	80 µg	TET80	≥ 28	23-27	15-22	≤ 14	≤ 4	4-12	16-64	> 64
a) b)	Ticarcillin	75 µg	TICAR	≥ 28	21-27	10-20	≤ 9	≤ 16	32-128	256	> 256
b)	Ticarcillin+ Clavulanate	75+15 µg	TI+CL	≥ 28	21-27	10-20	≤ 9	≤ 16	32-128	256	> 256
	Tigecycline	15 µg	TIG15	≥ 25	-	-	≤ 24	≤ 1	-	-	> 2
m)	Tobramycin	40 µg	TOBRA	≥ 26	23-25	15-22	≤ 14	≤ 4	6-8	12-64	> 64
	Trimethoprim (U)	5.2 µg	TRIME	≥ 22	19-21	13-18	≤ 12	≤ 4	6-8	12-16	> 16
	Trimethoprim+ Sulfa	5.2+240µg	TR+SU	≥ 28	23-27	15-22	≤ 14	≤ 1,6	1,6-3,2	3,2-12,8	> 12,8
e)	Vancomycin	5 µg	VAN.5	≥ 16	-	-	≤ 15	≤ 2	-	-	> 4
	Enterococcus spp. (Mueller-Hinton)			≥ 15	14-13	-	≤ 12	≤ 4	6-16	-	> 16
s)	2+18 t. prædiffusion (Staphylococcus spp.)			-	-	-	< 22	-	-	-	VISA/GISA
	Enterococcus spp. (M-H)			-	-	-	< 16	-	-	-	VRE

Bemærkninger til aflæsningsskemaet:

a) svares 0 for penicillin-resistente stafylokokker.

b) svares 0 for methicillin-resistente stafylokokker.

c) Ved resistensbestemmelse af Pseudomonas, samt *St. maltophilia* og *Burkholderia cepacia* anvendes:
3 ≥ 30 mm
2 20-29 mm
0 ≤ 19 mm

d) Streptokokker/enterokokker udviser intrinsisk resistens overfor aminoglykosider og bør kun testes for HLR (high level resistens) overfor Gentamicin 40 µg og Streptomycins 100 µg Neo-Sensitabs.

Gentamicin 40 µg: zone < 20 mm HLR (MIC > 500 µg/ml)
Streptomycins 100 µg: zone < 20 mm HLR (MIC > 1000 µg/ml)

e) For enterokokker anvendes Vancomycin 5 µg og Mueller-Hinton Agar (uden blod) med kraftig inokulum McFarland 0.5). På Dansk Blod Agar kan man ikke påvise VRE (van B).
Aflæsning: S ≥ 15 mm, I: 14-13 mm, R ≤ 12 mm. Gælder også for 2+18 t. prædiffusion.

f) Ved testning af pneumokokker med Oxacillin 1 µg anvendes kraftig inokulum (McFarland 0.5).
Aflæsning: S ≥ 20 mm, I/R ≤ 19 mm.
Hvis væksten på pladen kun er semikonfluerende anvendes:
S ≥ 25 mm, I/R ≤ 24 mm.

g) Ved testning af Methicillin med *Staph. aureus* anvendes: S ≥ 28 mm, R ≤ 27 mm. For Koagulase-negative stafylokokker (screening) anvendes kraftig inokulum (McFarland 0.5) og ≥ 28 mm, R ≤ 27 mm (Methicillin Neo-S). Hvis stammen (KNS) er multi-resistent og methicillin-følsom på DBA, testes den med Oxacillin 1 µg på Mueller-Hinton Agar (uden blod) og kraftigt inokulum (McFarland 0.5): S ≥ 18 mm (MIC ≤ 0.25 µg/ml). R ≤ 17 mm (MIC ≥ 0.5 µg/ml). Breakpoints fra NCCLS, **M2 A7**, 2000. Cefoxitin må testes sideløbende.

h) Enterobacter og *Proteus* spp. bør rapporteres **R** mod Nitrofurantoin, uanset zonestørrelse.

i) Bemærk **nye MIC** breakpoints for Kinoloner (skandinavisk model). Tidligere MIC breakpoints var fra CLSI.

j) *P. aeruginosa*, *St. maltophilia* samt *Proteus* spp. bør rapporteres **R** mod Tetracyclines Neo-Sensitabs, uanset zonestørrelse. *P. aeruginosa* bør også rapporteres R mod Chlormaphenicol og Streptomycin.

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k) Cefoxitin 60 µg og 10 µg er bedst til påvisning af methicillin-resistente staphylococcer. Med Cefoxitin 60 µg: *S. aureus* med zoner < 28 mm er mecA positive. For coag. neg. staph. anvendes < 32 mm. Med Cefoxitin 10 µg: *S. aureus* med zoner < 22 mm (mec A pos.). Coag. neg. staph: zoner < 27 mm (mec A pos.).

l) Klebsiella og Enterobacter rapporteres altid **R** mod ampicillin.

m) Staphylokokker resistente overfor gentamicin bør rapporteres **R** mod netilmicin og tobramycin.

n) Nalidixan er en god screening til påvirkning af nedsat følsomhed overfor kinoloner hos *Salmonella* spp. Stammer resistente overfor Nalidixan Neo-S (zone < 26 mm) viser nedsat følsomhed ovefor kinoloner (CIPRO MIC ≥ 0,125 µg/ml). (1)

o) *E. coli*/Klebsiella/Salmonella stammer der udviser zoner ≤ 23 mm overfor Cefpodoxime Neo-Sensitabs, producerer formodentlig ESBL enzymer. Til bekræftelse, test Ceftazidime+Clavulanate og Cefepime+Clavulanate mod Ceftazidime/Cefepime Neo-Sensitabs (se side 13.9.1).

p) Mecillinam Neo-Sensitabs kan bruges som screening for beta-lactamase produktion hos staphylococcer. Mecillinam resistente staphylococcer er også penicillin resistente (beta-lactamase produktion). Mecillinam zoner > 22 mm indicerer penicillin følsomhed hos *S. aureus* (2).

q) Til påvisning af hVISA/VISA stammer, se afsnittet i User's Guide "Detection of strains with decreased susceptibility to Vancomycin" side 86.

r) Ertapenem **må ikke testes** over for *P. aeruginosa* på Dansk Blod Agar, idet det kan resultere i falsk følsomme resultater.

s) 2+18 timers prædiffusionsteknikken er beskrevet på side 18.

Referencer:

1) Dragsted U.B. et al.: Relapse of multiresistant *Salmonella Tiphi* after combined therapy with ciprofloxacin and ceftriaxone. Eur. Soc. Clin. Microbiol. Infect. Dis. **6**, 167-8, 2000.
2) Bruun B., Gahrn-Hansen B: Mecillinam susceptibility as an indicator of betalactamase production in *Staphylococcus aureus.* Clin. Microbiol. & Infect. **8**, 122-124, 2002.
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10.5.1 Interpretations According to MIC Breakpoints of the Danish Reference Group for Susceptibility Testing

Vc

Aflæsningsskema for hurtigvoksende bakterier. Breakpoints ifølge DSKM Referencegruppe for antibiotika-resistens (DK). Substrat: DBA. Inoculum: Semikonfluerende vækst.

				Zone diameter i mm			Break-points MIC µg/ml	
NEO-SENSITABS	STYRKE	KODE	S	I	R	S	R
	Penicillin Low	5 µg	PEN.L					
l) m)	*Staphylococcus* spp.			≥ 31	30-29	≤ 28	≤ 0,12	Penicillinase
	Enterococcus spp.			≥ 26	25-11	≤ 10	≤ 0,25	> 4
l)	Oxacillin	1 µg	OXA.1					
	S. aureus og *S. lugdunensis*			≥ 16	-	< 16	≤ 1	> 1
	Coag. neg. staph.			≥ 20	-	< 20	≤ 0,25	> 0,5
g)	Cloxacillin	500 µg	CL500	Påvisning af plasmid AmpC				
l) m)	Ampicillin	33 µg	AMP33					
a)	*Enterobacteriaceae*			≥ 32	31-19	≤ 18	≤ 1	> 8
	Enterococcus spp.			≥ 26	25-19	≤ 18	≤ 2	> 8
	Mecillinam	33 µg	MECIL					
	Enterobacteriaceae			≥ 30	29-21	≤ 20	≤ 1	> 8
l) n) p)	Staphylococci (penicillin)			≥ 23	-	≤ 22	Påvisning af penicilinresistens	
m)	Piperacillin	100 µg	PIPRA					
	Enterobacteriaceae			≥ 28	27-24	≤ 23	≤ 16	> 16
	Pseudomonas spp./*Acinetobacter* spp.			≥ 28	27-24	≤ 23	≤ 16	> 16
i)	Aztreonam	30 µg	AZTRM					
	Enterobacteriaceae			≥ 34	33-29	≤ 28	≤ 0,5	> 1
n)	Cefuroxime	60 µg	CEFUR					
	Enterobacteriaceae			≥ 26	25-23	≤ 22	≤ 8	> 8
i) n)	Cefotaxime	30 µg	CFTAX					
	Enterobacteriaceae			≥ 34	33-29	≤ 28	≤ 0,5	> 1
i) n)	Ceftriaxone	30 µg	CETRX					
	Enterobacteriaceae			≥ 34	33-29	≤ 28	≤ 0,5	> 1
n)	Ceftazidime	30 µg	CEZDI					
	Enterobacteriaceae			≥ 28	27-24	≤ 23	≤ 2	> 4
	Pseudomonas spp./*Acinetobacter* spp.			≥ 23	22-21	≤ 20	≤ 8	> 8
f)	Ceftazidime+ Clav.	30+10 µg	CZ+CL	Påvisning af ESBL				
i)	Cefepime	30 µg	CFEPM	≥ 34	33-29	≤ 28	≤ 0,5	> 1
e) f)	Cefpodoxime	30 µg	CFPOX			≤ 23	-	Screen ESBL
	Cefpodoxime	10 µg	CFD10	-	-	< 20	-	Screen ESBL
f)	Cefepime+Clavulanate	30+10 µg	CP+CL	Påvisning af ESBL				
	Imipenem	15 µg	IMIPM					
	Enterobacteriaceae			≥ 28	27-19	≤ 18	≤ 1	> 8
	Pseudomonas spp.			≥ 26	25-21	≤ 20	≤ 4	> 8
	Acinetobacter spp.			≥ 28	27-19	≤ 18	≤ 1	> 8
h)	Imipenem+EDTA	15+750µg	IM+ED	Påvisning af Metallo-beta-lactamases				
	Meropenem	10 µg	MEROP					
	Enterobacteriaceae			≥ 34	33-17	≤ 16	≤ 0,12	> 8
	Pseudomonas spp.			≥ 30	29-21	≤ 20	≤ 2	> 8
	Acinetobacter spp.			≥ 30	29-21	≤ 20	≤ 1	> 8

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	NEO-SENSITABS	STYRKE	KODE	Zone diameter i mm S	I	R	Break-points MIC µg/ml S	R
j2)	Ertapenem	10 µg	ETP10					
	Enterobacteriaceae			≥ 30	29-26	< 26	≤ 0.5	> 1
	Cefoxitin	**60 µg**	CFOXT					
l)	*S. aureus* og *S. lugdunensis*			≥ 30	29-28	< 28	Oxa S	Mec A pos.
	- with MH agar:			≥ 28	27-26	< 26	Oxa S	Mec A pos.
l)	Coag. neg. staph.			≥ 34	33-32	< 32	Oxa S	Mec A pos.
	- with MH agar:			≥ 32	31-30	< 30	Oxa S	Mec A pos.
	Cefoxitin	**10 µg**	CFO10					
l)	*S. aureus* og *S. lugdunensis*			≥ 22	-	< 22	Oxa S	Mec A pos.
l)	Coag. neg. staph.			≥ 27	26-22	≤ 21	Oxa S	Mec A pos.
Aminoglycosider								
	Amikacin	40 µg	AMIKA					
	Enterobacteriaceae			≥ 26	25-23	≤ 22	≤ 4	> 8
	Pseudomonas spp.			≥ 26	25-23	≤ 22	≤ 8	> 8
	Acinetobacter spp.			≥ 26	25-23	≤ 22	≤ 4	> 4
	Staphylococcus spp.			Test Kanamycin			≤ 4	> 8
s)	Gentamicin	40 µg	GEN40					
	Enterobacteriaceae / Acinetobacter spp.			≥ 28	27-24	≤ 23	≤ 2	> 2
	Pseudomonas spp.			≥ 28	27-24	≤ 23	≤ 4	> 4
q)	*Staphylococcus aureus*			≥ 30	29-27	≤ 26	≤ 1	> 1
q)	Coag. neg. staph.			≥ 34	-	≤ 33	≤ 1	> 1
	Netilmicin	40 µg	NETIL					
	Enterobacteriaceae / Acinetobacter spp.			≥ 28	27-24	≤ 23	≤ 2	> 2
	Pseudomonas spp.			≥ 28	27-24	≤ 23	≤ 4	> 4
q)	*Staphylococcus aureus*			≥ 30	29-27	≤ 26	≤ 1	> 1
q)	Coag. neg. staph.			≥ 34	-	≤ 33	≤ 1	> 1
	Tobramycin	40 µg	TOBRA					
	Enterobacteriaceae / Acinetobacter spp.			≥ 28	27-24	≤ 23	≤ 2	> 2
	Pseudomonas spp.			≥ 28	27-24	≤ 23	≤ 4	> 4
q)	*Staphylococcus aureus*			≥ 30	29-27	≤ 26	≤ 1	> 1
q)	Coag. neg. staph.			≥ 34	-	≤ 33	≤ 1	> 1
	Kanamycin	100 µg	KANAM					
	Staphylococcus spp.			≥ 26	25-24	≤ 23	≤ 4	>16
s)	Streptomycin	100 µg	ST100					
	Staphylococcus spp.			≥ 28	-	≤ 27	≤ 8	>16
	Erythromycin	78 µg	ERYTR					
	Staphylococcus spp.			≥ 30	-	≤ 29	≤ 0,5	> 0,5
	Clindamycin	25 µg	CLIND					
o)	*Staphylococcus* spp.			≥ 32	31-28	≤ 27	≤ 0,5	> 2
	Fucidin	100 µg	FUCID					
	Staphylococcus spp.			≥ 25	-	≤ 24	≤ 0,5	> 0,5
	Chloramphenicol	60 µg	CLR60					
	Enterobacteriaceae			≥ 26	-	< 26	≤ 8	> 16
	Staphylococcus spp.			≥ 26	-	< 26	≤ 8	> 16
j)	Tetracyclines	80 µg	TET80					
	Staphylococcus spp.			≥ 32	31-29	≤ 28	≤ 2	> 2
*)	Tigecycline	15 µg	TIG15	≥ 25	-	≤ 24	≤ 1	> 2
	Rifampicin	30 µg	RIFAM					
	Staphylococcus spp.			≥ 35	34-31	≤ 30	≤ 1	> 2
	Enterococcus spp.			≥ 28	-	< 28	≤ 1	> 2
	Linezolid	30 µg	LINEZ					
	Staphylococcus spp.			≥ 27	26-23	≤ 22	≤ 4	> 4
	Enterococcus spp.			≥ 26	25-23	≤ 22	≤ 4	> 4

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	NEO-SENSITABS	STYRKE	KODE	Zone diameter i mm S	I	R	Break-points MIC µg/ml S	R
	Vancomycin	5 µg	VAN.5	≥ 16	-	< 16	≤ 2	> 4
h2)	2+18 t prædiffusion (BHI+blod)							
r)	*Staphylococcus* spp.			-	-	< 21	-	VISA/GISA
t)	*Enterococcus* spp. (MH)			-	-	< 16	-	VRE
	Teicoplanin	30 µg	TPN30					
h2)	2+18 t prædiffusion (BHI+blod)			-	-			VISA/GISA
r)	*Staphylococcus* spp.			-	-	< 20	-	VISA/GISA
t)	*Enterococcus* spp. (MH)			-	-	< 16	-	VRE
	Daptomycin	30 µg	DAPCa					
h2)	2+18 t prædiffusion							
	Staphylococcus spp.			≥ 22	-	-	≤ 1	-
	Enterococcus spp.			≥ 12	-	-	≤ 4	-
	Colistin	10 µg	CO.10					
h2)	2+18 t prædiffusion			≥ 15	-	< 15	≤ 2	> 2
	Ciprofloxacin	10 µg	CIP10					
d)	*Enterobacteriaceae*			≥ 34	33-23	≤ 22	≤ 0,06	>1
	Pseudomonas spp./*Acinetobacter* spp.			≥ 28	27-23	≤ 22	≤ 1	>1
	Staphylococcus spp.			≥ 24	23-18	≤ 17	≤ 1	>4
	Norfloxacin	10 µg	NORFX				Nedsat følsomhed	
	Staphylococcus spp.			-	-	< 20	for kinoloner	
	Moxifloxacin	5 µg	MOXIF					
	Staphylococcus spp.			≥ 29	28-26	≤ 25	≤ 0,25	> 0,5
b)	Nitrofurantoin (U)	260 µg	NITRO	≥ 26	-	< 26	≤ 32	> 32
	Trimethoprim	5,2 µg	TRIME	≥ 22	21-19	≤ 18	≤ 2	> 4
	Trimethoprim+Sulfa	5,2+240µg	TR+SU	≥ 28	27-24	≤ 23	≤ 16	> 32
	Sulphonamides (U)	240 µg	SULFA	≥ 22	-	< 22	≤ 512	> 512
	Nalidixan (U)	130 µg	NALID	≥ 28	27-24	≤ 23	≤ 8	≥ 32
c)	*Enterobacteriaceae*			-	-	< 26	Nedsat følsomhed for kinoloner	
	Staphylococcus spp. (undtagen *S. saprophyticus*)			-	-	ingen zone	Muligvis nedsat følsomhed for kinoloner	
	Novobiocin (U)	5 µg	NOVO5	≥ 16	-	< 16	≤ 2	> 2

*) Tentative

Bemærkninger til aflæsningsskemaet:

Enterobacteriaceae

a) Klebsiella og Enterobacter rapporteres altid **R** mod ampicillin.

b) Enterobacter og *Proteus* spp. bør rapporteres **R** mod Nitrofurantoin, uanset zonestørrelse.

c) Nalidixan er en god screening til påvisning af nedsat følsomhed overfor kinoloner hos *Salmonella* spp. Stammer resistente overfor Nalidixan Neo-S (zone < 26 mm) viser nedsat følsomhed ovefor kinoloner (CIPRO MIC ≥ 0,125 µg/ml). (1)

d) Bemærk **nye MIC** breakpoints for Kinoloner (skandinavisk model). Tidligere MIC breakpoints var fra CLSI. Bemærk at EUCAST anbefaler en følsom breakpoint for ciprofloxacin med Enterobacteriaceae på 0,5 µg/ml (svarende til en zone på 28 mm).

e) *E. coli*/Klebsiella/Salmonella stammer der udviser zoner ≤ 23 mm overfor Cefpodoxime Neo-Sensitabs, producerer formodentlig ESBL enzymer. Til bekræftelse, test Ceftazidime+Clavulanate og Cefepime+Clavulanate mod Ceftazidime/Cefepime Neo-Sensitabs (se side 13.9.1).

f) Til påvisning af ESBL, se kapitel 16.1: "Extended-spectrum beta-lactamases (ESBL)".

g) Til påvisning af plasmid-mediated Amp C beta-lactamases, se kapitel 16.4: "Plasmid-mediated AmpC beta-lactamases".

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h) Til påvisning af metallo-beta-lactamaser og andre carbapenemaser, se kapitel 16.6: "Carbapenemases".

h2) **Prædiffusionsmetode**. Er beskrevet i detaljer på side 18. Kan med fordel anvendes til påvisning af Colistin-resistente *P. aeruginosa* / *A. baumannii*, Daptomycinresistente staphylococci/enterococci, samt påvisning af VISA/GISA stammer.

Non-fermenters

i) Ved resistensbestemmelse af Pseudomonas, samt *St. maltophilia* og *Burkholderia cepacia* anvendes:
S: ≥ 30 mm
I: 29-20 mm
R: ≤ 19 mm

j) *P. aeruginosa*, *St. maltophilia* samt *Proteus* spp. bør rapporteres **R** mod Tetracyclines Neo-Sensitabs, uanset zonestørrelse.

j2) Ertapenem **må ikke testes** over for *P. aeruginosa* på Dansk Blod Agar, idet det kan resultere i falsk følsomme resultater.

k) Til resistensbestemmelse af *Acinetobacter* spp., *St. maltophilia* og *B. cepacia* se kapitel 15.10.

Staphylococcer

l) Staphylococcer og beta-lactam testning:
Til påvisning af beta-lactamase anvendes Penicillin Low Neo-Sensitabs. I tvivlstilfælde kan resultaterne kontrolleres med Mecillinam Neo-Sensitabs.
Til påvisning af methicillin/oxacillin resistens anvendes surrogattestning med Cefoxitin 60 µg eller 10 µg samt Oxacillin 1 µg Neo-Sensitabs.
Med Cefoxitin 60 µg gælder følgende:
S. aureus og *S. lugdunensis* S ≥ 30 mm, I: 29-28 og R < 28 mm
Koagulase negative staph. S ≥ 34 mm, I: 33-32 og R < 32 mm
Med Cefoxitin 10 µg gælder:
S. aureus og *S. lugdunensis* S ≥ 22 mm, R < 22 mm
Koagulase negative staph. S ≥ 27 mm, I: 26-22 og R ≤ 21 mm
Staphylococcer resistente overfor cefoxitin må betragtes som resistente overfor alle beta-lactam antibiotika uanset zone størrelse.

m) svares 0 for penicillin-resistente stafylokokker.

n) svares 0 for methicillin-resistente stafylokokker.

o) Til påvisning af clindamycin resistens hos erythromycin resistente staphylokokker, se afsnittet "Double tablet induction test (D zone-test)" side 89.

p) Mecillinam Neo-Sensitabs kan bruges som screening for beta-lactamase produktion hos staphylococcer. Mecillinam resistente staphylococcer er også penicillin resistente (beta-lactamase produktion). Mecillinam zoner > 22 mm indicerer penicillin følsomhed hos *S. aureus* (2).

q) Staphylokokker resistente overfor gentamicin bør rapporteres **R** mod netilmicin og tobramycin.

r) Til påvisning af hVISA/VISA stammer, se afsnittet: "Detection of strains with decreased susceptibility to vancomycin (VISA/GISA, hVISA)" side 86.

Enterococcer

s) Streptokokker/enterokokker udviser intrinsisk resistens overfor aminoglykosider og bør kun testes for HLR (high level resistens) overfor Gentamicin 40 µg og Streptomycins 100 µg Neo-Sensitabs.
Gentamicin 40 µg: zone < 20 mm HLR (MIC > 500 µg/ml)
Streptomycins 100 µg: zone < 20 mm HLR (MIC > 1000 µg/ml)

t) For enterokokker anvendes Vancomycin 5 µg og Mueller-Hinton Agar (uden blod) med kraftig inokulum McFarland 0.5).
Aflæsning: S ≥ 15 mm, I: 14-13 mm, R ≤ 12 mm
Man kan ikke påvise VanB resistente enterococcer på DBA anvend derfor MH agar.

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Referencer:

1) Dragsted U.B. et al.: Relapse of multiresistant *Salmonella Typhi* after combined therapy with ciprofloxacin and ceftriaxone. Eur. Soc. Clin. Microbiol. Infect. Dis. **6**, 167-8, 2000.
2) Bruun B., Gahrn-Hansen B: Mecillinam susceptibility as an indicator of betalactamase production in *Staphylococcus aureus.* Clin. Microbiol. & Infect. **8**, 122-124, 2002.
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4) Klaringsrapport. Dansk Selskab for Klinisk Mikrobiologisk Referencegruppe vedrørende Antibiotika. Resistensbestemmelse. 20.04.2004.

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Vd

Streptococcus spp., *S. pneumoniae*, *Haemophilus* spp., *Moraxella catarrhalis*, *Corynebacterium* spp., *Listeria* spp.[n)]
Breakpoints ifølge DSKM Referencegruppe for antibiotikaresistens (DK).
Substrat: DBA + 5% CO_2 (*Haemophilus* spp. Chokolade Agar)
Inoculum: Semikonfluerende vækst.

		NEO-SENSITABS	STYRKE	KODE	Zone diameter i mm S	I	R	Break-points MIC µg/ml S	R
k)		Penicillin Low	5 µg	PEN.L					
		Streptococcus spp.			≥ 26	25-21	≤ 20	≤ 0,25	> 1
		Haemophilus spp.			≥ 20	19-18	< 18	≤ 1	> 1
		Moraxella catarrhalis			Test beta laktamase			-	Penase
		Corynebacterium spp./ *Listeria* spp.			≥ 26	25-21	≤ 20	≤ 0,25	> 1
j)	k)	Oxacillin	1 µg	OXA.1					
c)		*Streptococcus* spp. (penicillin)			≥ 17	< 17	< 17	≤ 0,12	MIC
e)		*S. pneumoniae* (penicillin)			≥ 24	≤ 23	≤ 23	≤ 0,06	MIC
		Amoxycillin+Clav.	30+15 µg	AM+CL					
		Haemophilus spp.			≥ 30	29-17	≤ 26	≤ 2	> 4
		Ampicillin	**2,5 µg**	AMP.L					
		Streptococcus spp.			≥ 24	23-19	≤ 18	≤ 0,25	> 1
a)		*Haemophilus* spp.			≥ 22	21-19	≤ 18	≤ 0,5	> 0,5
	m)	*Moraxella catarrhalis*			Test beta laktamase			-	Penase
		Ampicillin	**33 µg**	AMP33					
		Streptococcus spp.			≥ 36	35-29	≤ 28	≤ 0,25	1
		Haemophilus spp. (test Amp. 2,5)			≥ 34	33-31	≤ 30	≤ 0,5	> 0,5
		Corynebacterium spp.			≥ 36	35-29	≤ 28	≤ 0,25	> 1
		Listeria spp.			≥ 34	33-31	≤ 30	≤ 0,5	> 1
		Cefuroxime	60 µg	CEFUR					
		Haemophilus spp.			≥ 34	33-29	≤ 28	≤ 2	> 2
		Moraxella catarrhalis			≥ 34	33-29	≤ 28	≤ 2	> 2
		Corynebacterium spp.			≥ 40	39-31	≤ 30	≤ 0,12	> 2
		Cefotaxime	30 µg	CFTAX					
		Streptococcus spp.			≥ 38	37-27	≤ 26	≤ 0,12	> 2
f)		*S. pneumoniae*			Test ceftizoxime som surrogat			≤ 0,12	> 1
		Haemophilus spp.			≥ 38	37-29	≤ 28	≤ 0,12	> 1
		Ceftizoxime	30 µg	CEZOX					
f)		*S. pneumoniae*			≥ 32	-	< 32	≤ 0,5	MIC 3. gen. cepha.
		Ceftriaxone	30 µg	CETRX					
		Streptococcus spp.			≥ 38	37-27	≤ 26	≤ 0,12	> 2
f)		*S. pneumoniae*			Test ceftizoxime som surrogat			≤ 0,12	> 1
		Haemophilus spp.			≥ 38	37-29	≤ 28	≤ 0,12	> 1
		Aztreonam	30 µg	AZTRM					
		Haemophilus spp.			≥ 40	39-35	≤ 34	≤ 0,5	> 1
		Imipenem	15 µg	IMIPM					
		Streptococcus spp.			≥ 36	35-24	≤ 23	≤ 0,06	> 2
		S. pneumoniae			≥ 36	35-29	≤ 28	≤ 0,06	> 0,5
		Haemophilus spp.			≥ 34	33-29	≤ 28	≤ 1	> 2

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	NEO-SENSITABS	STYRKE	KODE	Zone diameter i mm S	I	R	Break-points MIC µg/ml S	R
	Meropenem	10 µg	MEROP					
	Streptococcus spp.			≥ 36	35-24	≤ 23	≤ 0,06	> 2
	S. pneumoniae			≥ 36	35-27	≤ 26	≤ 0,06	> 0,5
	Haemophilus spp.			≥ 34	33-29	≤ 28	≤ 1	> 2
	Ertapenem	10 µg	ETP10					
	Streptococcus spp.			≥ 30	29-26	< 26	≤ 0.5	> 1
	S. pneumoniae			≥ 30	29-26	< 26	≤ 0.5	> 1
	Haemophilus spp./Moraxella catarrhalis			≥ 30	29-26	< 26	≤ 0.5	> 1
	Anaerobes			≥ 32	31-28	< 28	≤ 1	> 1
	Erythromycin	78 µg	ERYTR					
d) g)	*Streptococcus* spp./*S. pneumoniae*			≥ 28	-	≤ 27	≤ 0,5	> 0,5
	Moraxella catarrhalis			≥ 32	31-22	≤ 21	≤ 0,5	> 4
	Corynebacterium spp./*Listeria* spp.			≥ 32	31-22	≤ 21	≤ 0,5	> 4
	Clindamycin	25 µg	CLIND					
d)	*Streptococcus* spp./*S. pneumoniae*			≥ 32	31-27	≤ 26	≤ 0,5	> 2
	Chloramphenicol	60 µg	CLR60					
	S. pneumoniae			≥ 26	25-23	≤ 22	≤ 8	> 16
	Haemophilus spp.			≥ 34	33-31	≤ 30	≤ 2	> 4
	Tetracyclines	80 µg	TET80					
	Streptococcus spp./*S. pneumoniae*			≥ 28	27-24	≤ 23	≤ 2	> 2
	Haemophilus spp./*Moraxella catarrhalis*			≥ 28	27-24	≤ 23	≤ 2	> 2
	Rifampicin	30 µg	RIFAM					
	Streptococcus spp./*S. pneumoniae*			≥ 32	31-29	≤ 28	≤ 1	> 2
	Linezolid	30 µg	LINEZ					
	Streptococcus spp./*S. pneumoniae*			≥ 28	27-24	≤ 23	≤ 2	> 4
	Vancomycin	5 µg	VAN.5					
	Streptococcus spp./*S. pneumoniae*			≥ 18	17-16	≤ 15	≤ 2	> 2
	Corynebacterium spp. /*Listeria* spp.			≥ 18	17-16	≤ 15	≤ 4	> 4
	Teicoplanin	60 µg	TEICO					
	Streptococcus spp./*S. pneumoniae*			≥ 24	23-17	≤ 16	≤ 0,5	> 4
	Corynebacterium spp. /*Listeria* spp.			≥ 18	17-16	≤ 15	≤ 4	> 4
i) l)	Ciprofloxacin	10 µg	CIP10					
b)	*Haemophilus* spp.			≥ 36	35-29	≤ 28	≤ 0,12	> 0,25
	Moraxella catarrhalis			≥ 36	35-29	≤ 28	≤ 0,12	> 0,25
i) l)	Nalidixan	130 µg	NALID				Nedsat følsomhed for kinoloner	
b)	*Haemophilus* spp.			-	-	< 28		
	Norfloxacin	10 µg	NORFX				Nedsat følsomhed for kinoloner	
	Pneumococcus spp.			-	-	< 16		
i) l)	Moxifloxacin	5 µg	MOXIF					
h)	*Streptococcus* spp./*S. pneumoniae*			≥ 26	25-21	≤ 20	≤ 1	> 2
b)	*Haemophilus* spp./*Moraxella catarrhalis*			≥ 33	32-29	≤ 28	≤ 0,25	> 0,5
	Trimethoprim	5,2 µg	TRIME					
	Streptococcus spp.			≥ 22	21-19	≤ 18	≤ 2	> 4
	Trimethoprim+Sulfa	5,2+240µg	TR+SU					
	Streptococcus spp./*S. pneumoniae*			≥ 32	31-27	≤ 26	≤ 16	> 32
	Haemophilus spp./ *Listeria* spp.			≥ 32	31-27	≤ 26	≤ 16	> 32

Bemærkninger:

Haemophilus

a) Beta-lactamase negative ampicillin resistente (BLNAR) påvises bedst med Ampi 2,5 µg. BLNAR isolater må betragtes som resistente over for amox, amox+clav samt 1. og 2. generations cefalosporiner, uanset zonestørrelse.

b) Nalidixanresistente stammer udviser nedsat følsomhed for ciprofloxacin (MIC > 0,125 µg/ml) og må betragtes som nedsat følsomme over for alle kinoloner.

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Streptococci

c) Oxacillin 1 µg kan anvendes til påvisning af viridans streptococcer med nedsat følsomhed over for penicillin. Se speciel aflæsning

d) Til påvisning af inducerbar clindamycinresistens hos streptococcer se afsnittet "Double tablet induction test (D Zone-test)" side 117.

S. pneumoniae

e) Oxacillin 1 µg anvendes til påvisning af stammer med nedsat følsomhed over for penicillin. Penicillinresistente isolater fra spinalvæske må betragtes som resistente over for ampicillin/amoxycillin, amoxycillin+clav samt 1. og 2. generations cefalosporiner.
Er væksten semikonfluerende, anvendes S ≥ 24 mm og I/R ≤ 23 mm.
Er væksten konfluerende, anvendes S ≥ 20 mm og I/R ≤ 19 mm

f) Cefotaxime og Ceftriaxone må ikke testes over for pneumococcer ved diffusionsmetoder. Anvend surrogat-testen Ceftizoxime.

g) Erythromycin. Aflæsningsresultatet gælder også for Azithromycin og Clarithromycin.

h) Screening af pneumococcer med nedsat følsomhed overfor kinoloner kan udføres ved at anvende Norfloxacin Neo-S. Hvis hæmningszonen er < 16 mm (eller MIC > 16 µg/ml), er der stor sandsynlighed for udvikling af resistente mutanter in vivo.

Gonococcer

i) Ciprofloxacinresistente gonococcer (anvend Nalidixan som surrogat-test) må betragtes som resistente overfor alle kinoloner.

j) Oxacillin 1 µg kan anvendes til påvisning af beta-lactamase negative gonococcer med nedsat følsomhed over for penicillin (kromosomal resistens). Se speciel aflæsning.

Meningococcer

k) Oxacillin 1 µg kan anvendes til screening af meningococcer med nedsat følsomhed over for penicillin (kromosomal resistens). Se speciel aflæsning.

l) Nalidixan er anvendelig til screening af stammer med nedsat følsomhed over for kinoloner.

Moraxella catharralis

m) Beta-lactamaseproduktion hos *M. catharralis* kan påvises ved at teste både Amoxycillin og Amoxycillin+Clav Neo-S. Beta-lactamase positive stammer vil udvise zoner > 5 mm med Amox+Clav i forhold til Amoxycillin alene.

***Listeria* spp.**

n) Listeria er intrinsisk resistent overfor: cephalosporiner, aztreonam, clindamycin, fosfomycin, colistin og nalidixan og må derfor ikke testes med ovennævnte antibakterielle midler.

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10.6 VI - Interpretation according to MIC Breakpoints of SFM (France)

VI-a

Rapidly growing bacteria *. Interpretation according to the MIC break-points recommended by the "Comité de l'Antibiogramme de la Societé Française de Microbiologie" (Jan. 2006)

	NEO-SENSITABS	POTENCY	CODE	Zone diameter in mm S	I	R	Concentrations critiques S	R
	Amikacin	40 µg	AMIKA	≥ 20	19-17	≤ 16	≤ 8	> 16
a)	Amoxycillin	30 µg	AMOXY	≥ 23	22-20	≤ 19	≤ 4	> 16
c)	Amoxycillin+Clav.	30+15 µg	AM+CL	≥ 23	22-20	≤ 19	≤ 4/2	> 16/2
a)	Ampicillin	33 µg	AMP33					
	Enterobacteriaceae			≥ 23	22-20	≤ 19	≤ 4	> 16
b)	Ampicillin+Sulbactam	30+30 µg	AM+SU	≥ 23	22-20	≤ 19	≤ 4/8	> 16/8
	Azithromycin	30 µg	AZITR	≥ 23	27-17	≤ 16	≤ 0.5	> 4
o) g)	Aztreonam	30 µg	AZTRM	≥ 25	24-21	≤ 20	≤ 4	> 32
	Bacitracin	40 U	BACIT	≥ 20	-	≤ 19	≤ 2	> 2
	Cefaclor	30 µg	CCLOR	≥ 25	24-21	≤ 2	≤ 2	> 8
	Cefadroxil	30 µg	CFDRO	≥ 20	19-17	≤ 16	≤ 8	> 32
o) g)	Cefepime	30 µg	CFEPM	≥ 23	22-20	≤ 19	≤ 4	> 32
	Cefepime+Clavulanate	30+10µg	CP+CL	Detection of ESBL				
g)	Cefixime	30 µg	CFFIX	≥ 28	27-24	≤ 23	≤ 1	> 2
g)	Cefotaxime	30 µg	CFTAX	≥ 23	22-20	≤ 19	≤ 4	> 32
	Cefotetan	30 µg	CFTTN	≥ 23	22-20	≤ 19	≤ 4	> 32
	Cefoxitin	60 µg	CFOXT	≥ 23	22-20	≤ 19	≤ 8	> 32
	S. aureus			≥ 26	-	≤ 25	Oxa S	Mec A pos.
	Coag. neg. staph.			≥ 30	-	≤ 29	Oxa S	Mec A pos.
g)	Cefpirome	30 µg	CFPIR	≥ 23	22-20	≤ 19	≤ 4	> 32
g) m)	Cefpodoxime (Scr. ESBL)	30 µg	CFPOX	≥ 24	-	≤ 23	≤ 4	> 4
	Cefsulodin	30 µg	CFSUL					
	Pseudomonas spp.			≥ 23	22-20	≤ 19	≤ 8	> 32
o) g)	Ceftazidime	30 µg	CEZDI	≥ 23	22-20	≤ 19	≤ 4	> 32
	Ceftazidime+Clav.	30+10µg	CZ+CL	Detection of ESBL				
g)	Ceftizoxime	30 µg	CEZOX	≥ 23	22-20	≤ 19	≤ 4	> 32
g)	Ceftriaxone	30 µg	CETRX	≥ 23	22-20	≤ 19	≤ 4	> 32
	Cefuroxime (parenteral)	60 µg	CEFUR	≥ 23	22-20	≤ 19	≤ 8	> 32
	Cefuroxime (oral)	60 µg	CEFUR	≥ 30	29-26	≤ 25	≤ 1	> 4
	Cephalexin	30 µg	CFLEX	≥ 20	19-17	≤ 16	≤ 8	> 32
	Cephalothin	66 µg	CLOTN	≥ 23	22-20	≤ 19	≤ 8	> 32
	Cephadrine	60 µg	CFRAD	≥ 23	22-20	≤ 19	≤ 8	> 32
	Chloramphenicol	60 µg	CLR60	≥ 25	24-21	≤ 20	≤ 8	> 16
	Ciprofloxacin	10 µg	CIP10	≥ 25	24-22	≤ 21	≤ 0.5	> 1
	P. aeruginosa + *Acinetobacter* spp.			≥ 23	22-20	≤ 19	≤ 1	> 2
	Clarithromycin	30 µg	CLARI	≥ 20	19-17	≤ 16	≤ 1	> 4
	Clindamycin	25 µg	CLIND	≥ 24	-	≤ 24	≤ 2	> 2
	Cloxacillin	500 µg	CL500	Detection of plasmid med. Amp C				
t)	Colistin	10 µg	CO.10					
**)	2+18 hours' prediffusion			≥ 15	14-11	≤ 10	≤ 2	> 2

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	NEO-SENSITABS	POTENCY	CODE	Zone diameter in mm S	I	R	Concentrations critiques S	R
	Daptomycin	30 µg	DAPCa					
**)	2+18 hours' prediffusion							
	Staphylococcus spp.			≥ 22	-	< 20	≤ 1	≥ 2
	Enterococcus spp.			≥ 12	-	< 11	≤ 4	≥ 8
	Doxycycline	80 µg	DOXYC	≥ 23	22-20	≤ 19	≤ 4	> 8
	Ertapenem	10 µg	ERTAP	≥ 23	22-21	≤ 20	≤ 2	> 4
	Erythromycin	78 µg	ERYTR	≥ 25	24-21	≤ 20	≤ 1	> 4
r)	Fosfomycin	70+40 µg	FOSFO	≥ 16	-	≤ 16	≤ 32	> 32
	Fucidin	100 µg	FUCID	≥ 28	27-21	≤ 20	≤ 2	> 16
	Gatifloxacin	5 µg	GATIF	≥ 21	20-18	≤ 17	≤ 1	≥ 2
n)	Gentamicin	**40 µg**	GEN40	≥ 24	23-21	≤ 20	≤ 2	> 4
	Staphylococcus spp.			≥ 26	-	< 26	≤ 1	> 1
	P. aeruginosa + *Acinetobacter* spp.			≥ 23	22-20	≤ 19	≤ 4	≥ 8
h)	Gentamicin (HNR)	**250 µg**	GN250	≥ 14	-	< 14	≤ 250	> 500
	Imipenem	15 µg	IMIPM	≥ 23	22-20	≤ 19	≤ 4	> 8
	Pseudomonas spp.			≥ 28	27-24	≤ 23	≤ 4	≥ 8
	Imipenem+EDTA	15+750µg	IM+ED	Detection of metallo-ß-lactamases				
	Isepamicin	30 µg		≥ 20	19-17	≤ 16	≤ 8	> 16
q)	Kanamycin	100 µg	KANAM	≥ 23	22-20	≤ 19	≤ 8	> 16
h)	Kanamycin (HNR)	500 µg	KA500	≥ 14	-	< 14	≤ 250	> 500
	Levofloxacin	5 µg	LEVOF	≥ 22	21-19	≤ 18	≤ 1	> 2
	Enterococcus spp.			≥ 21	20-17	≤ 16	≤ 1	> 4
	Lincomycin	19 µg	LINCO	≥ 26	25-23	≤ 22	≤ 2	> 8
	Linezolid	30 µg	LINEZ	≥ 28	27-26	≤ 25	≤ 2	> 4
	Staphyloccus spp/*Enterococcus* spp.			≥ 24	-	< 24	≤ 4	> 4
	Moxifloxacin	5 µg	MOXIF	≥ 24	23-21	≤ 20	≤ 0.5	> 1
	Enterococcus spp.			≥ 21	20-18	≤ 17	≤ 1	> 2
	Mecillinam (U)	33 µg	MECIL	≥ 23	22-20	≤ 19	≤ 2	> 8
	Meropenem	10 µg	MEROP	≥ 20	19-17	≤ 16	≤ 4	> 8
	Pseudomonas spp.			≥ 26	25-23	≤ 22	≤ 4	> 8
	Minocycline	80 µg	MINOC	≥ 23	22-20	≤ 19	≤ 4	> 8
l)	Mupirocin	10 µg	MUPIR	≥ 18	-	-	≤ 2	-
p)	Nalidixan (U)	130 µg	NALID	≥ 25	24-21	≤ 20	≤ 8	> 16
	Enterobacteriaceae			-	-	< 25	Reduced susceptibility to quinolones	
	Neomycin	120 µg	NEOMY	≥ 25	24-21	≤ 20	≤ 8	> 16
	Netilmicin	40 µg	NETIL	≥ 24	23-21	≤ 20	≤ 2	> 4
	P. aeruginosa + *Acinetobacter* spp.			≥ 23	22-20	≤ 19	≤ 4	> 8
	Nitrofurantoin (U)	260 µg	NITRO	≥ 23	22-20	≤ 19	≤ 32	> 128
	Norfloxacin (U)	10 µg	NORFX	≥ 25	24-23	≤ 22	≤ 0.5	> 1
	Ofloxacin	10 µg	OFLOX	≥ 25	24-22	≤ 21	≤ 0.5	> 1
	Oxacillin 1 µg	1 µg	OXA.1					
f)	*Staph. aureus*			≥ 16	-	< 16	≤ 2	> 2
	Coag. neg. staph.			≥ 20	-	< 20	≤ 0.25	> 0.5
	Oxacillin 5 µg	5 µg	OXA.5					
f)	*Staph. aureus*			≥ 20	-	< 20	≤ 2	> 2
	Oxolinic acid (U)	10 µg	OXOLI	≥ 18	17-15	≤ 14	≤ 2	> 4

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	NEO-SENSITABS	POTENCY	CODE	Zone diameter in mm S	I	R	Concentrations critiques S	R
	Pefloxacin	10 µg	PEFLX	≥ 23	22-17	≤ 16	≤ 1	> 4
	Penicillin Low	5 µg	PEN.L					
	Staphylococcus spp.			≥ 26	-	< 26	≤ 0.25	beta-lactamase
	Pipemidic acid (U)	30 µg	PIPEM	≥ 20	19-17	≤ 18	≤ 8	> 16
	Piperacillin	100 µg	PIPRA					
a)	Enterobacteriaceae			≥ 23	22-20	≤ 19	≤ 8	> 64
	Pseudomonas spp../*Acinetobacter* spp.			≥ 20	19-17	≤ 16	≤ 16	> 64
	Piperacillin+Tazobactam	100+10µg	PI+TZ					
e)	Enterobacteriaceae			≥ 23	22-20	≤ 19	≤ 8/4	> 64/4
e)	*Pseudomonas* spp../*Acinetobacter* spp.			≥ 20	19-17	≤ 18	≤ 16/4	> 64/4
	Polymyxins (colistin)	150 µg	CO150	≥ 20	-	≤ 19	≤ 2	> 2
	Pristinamycin	30 µg	PRIST	≥ 25	24-21	≤ 20	≤ 1	> 2
	Quinu/Dalfopristin	15 µg	SYN15	≥ 25	24-19	≤ 18	≤ 0.5	> 2
	Rifampicin	30 µg	RIFAM					
	Staphylococcus spp.			≥ 28	27-24	≤ 23	≤ 0.5	> 4
	- other			≥ 23	22-18	≤ 17	≤ 4	> 16
	Sparfloxacin	10 µg		≥ 23	22-20	≤ 19	≤ 1	> 2
	Spiramycin	200 µg	SPIRA	≥ 26	25-23	≤ 22	≤ 1	> 4
	Streptomycin	100 µg	ST100	≥ 25	24-21	≤ 20	≤ 8	> 16
h)	Streptomycin (HNR)	500 µg	ST500	≥ 14	-	≤ 14	≤ 250	> 500
	Sulphonamides (U)	240 µg	SULFA	≥ 23	22-20	≤ 19	≤ 100	> 350
k) s)	Teicoplanin	30 µg	TPN30	≥ 16	15-14	< 14	≤ 4	> 8
**)	2+18 hours' prediffusion			-	-	< 22	≤ 2	≥ 4 (VISA/GISA)
	Telithromycin	15 µg	TEL15	≥ 21	20-17	≤ 16	≤ 0.5	> 2
l)	Temocillin	30 µg	TEMOC	≥ 18	17-15	≤ 14	≤ 16	≥ 32
	Tetracyclines	80 µg	TET80	≥ 23	22-20	≤ 19	≤ 4	> 8
a)	Ticarcillin	75 µg	TICAR	≥ 23	22-20	≤ 19	≤ 16	> 64
d) o)	Ticarcillin+Clavulanate	75+15 µg	TI+CL	≥ 23	22-20	≤ 19	≤ 16/2	> 64/2
	Tigecycline	15 µg	TIG15	≥ 19	18-15	< 15	≤ 2	> 8
	Tobramycin	40 µg	TOBRA	≥ 24	23-21	≤ 20	≤ 2	> 4
	Staphylococcus spp.			≥ 26	-	< 26	≤ 1	> 1
	P. aeruginosa + *Acinetobacter* spp.			≥ 23	22-20	≤ 19	≤ 4	≥ 8
	Trimethoprim (U)	5.2 µg	TRIME	≥ 20	19-17	≤ 16	≤ 4	> 8
i)	Trimethoprim+Sulfa	5.2+240 µg	TR+SU	≥ 28	27-24	≤ 23	≤ 2/38	> 8/152
k) s)	Vancomycin	5 µg	VAN.5	≥ 16	-	-	≤ 4	> 8
**)	2+18 hours' prediffusion (staph.)			-	-	< 20	-	> 2 (VISA/GISA)
	2+18 hours' prediffusion (enterococci)			-	-	< 16	VRE	-
	Virginiamycin	30 µg	VIRGI	≥ 25	24-21	≤ 20	≤ 1	> 2

*) Enterobacteriaceae, *Pseudomonas* spp., *Acinetobacter* spp., *Staphylococcus* spp., *Enterococcus* spp.

**) Special technique for testing high molecular weight antimicrobials (colistin, daptomycin, teicoplanin, vancomycin): 2+18 hours' prediffusion method, permitting a good separation between susceptible and resistant strains. Description of the procedure on page 18.

Remarks:

a) *Klebsiella* spp. produces a natural low level beta-lactamase that inactivates amino-, carboxy- and ureido-penicillins. They may appear susceptible in vitro, but they should be reported as Intermediate to carboxy- and ureido-penicillins.

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b) Critical concentrations of ampicillin with a fixed concentration of sulbactam (8 µg/ml).

c) Critical concentrations of amoxycillin with a fixed concentration of clavulanic acid (2 µg/ml).

d) Critical concentrations of ticarcillin with a fixed concentration of clavulanic acid (2 µg/ml).

e) Critical concentrations of piperacillin with a fixed concentration of tazobactam (4 µg/ml).

f) For detecting methicillin/oxacillin resistance in staphylococci follow the instructions in User's Guide, chapter 13.1. Test Cefoxitin Neo-Sensitabs.
Strains resistant to oxacillin should be reported as resistant to all beta-lactams, even if they appear susceptible in vitro.

g) Strains of *Klebsiella* spp. and E. coli may be clinically resistant to cephalosporins and aztreonam therapy by producing ESBL (extended spectrum beta-lactamase). Read in chapter 16.1 of User's Guide, how to detect ESBL.

h) These high content Neo-Sensitabs are used to detect high level resistance to the aminoglycosides. Read in chapter 14.3, how to detect HLR (HNR).

i) The interpretation of results is valid for other combinations of Trimethoprim+Sulphonamide.

k) Strains showing inhibition zones smaller than the limit for susceptible, should be tested by an MIC method.

l) MIC breakpoints (concentrations critiques) not yet established by the Comité de l'Antibiogramme (SFM).

m) Strains showing zone < 23 mm with Cefpodoxime Neo-Sensitabs, should be suspected of producing ESBL (*E. coli*, Klebsiella, Salmonella).
For ESBL confirmatory tests see chapter 16.1.

n) Results with gentamicin and staphylococci are also valid for netilmicin. Staphylococci that are resistant to gentamicin, should be reported as resistant against both netilmicin and tobramycin (enzymes APH(2”)+AAC(6’)).

o) Synergism between TTC (TI + CL) and Aztreonam/Ceftazidime/Cefepime permits the detection of ESBL producing strains in *Ps. aeruginosa*.

p) Nalidixan is useful to detect strains with reduced susceptibility to quinolones in 1)Enterobacteriaceae (Nali zone < 25 mm) 2) *Haemophilus influenzae* (Nali zone < 25 mm) 3) Gonococci (Nali zone < 28 mm) and 4) *Vibrio cholerae* (Nali zone < 25 mm).

q) With staphylococci, the interpretation is valid for amikacin and isepamicin.

r) Do not take into account the presence of colonies inside the zone of inhibition. Resistant strains show homogeneous resistance.

s) For detection of VISA, GISA, hVISA strains, see chapter in User's Guide on detection of staphylococci with decreased susceptibility to vancomycin, page 86.

t) Zones tentative for 1 year.

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VI-b

Haemophilus spp., *S. pneumoniae*, *Streptococcus* spp., *N. gonorrhoeae*, *N. meningitidis*, *Campylobacter* spp. and anaerobes. Interpretation according to the MIC break-points recommended by the "Comité de l'Antibiogramme de la Societé Française de Microbiologie" (Jan. 2006) Inoculum, media and incubation conditions acc. to SFM (2006)

	NEO-SENSITABS	POTENCY	CODE	Zone diameter in mm S	I	R	Concentrations critiques S	R
	Ampicillin	**33 µg**	AMP33					
e)	*Haemophilus* spp.			-	-	< 32	-	> 1
	Campylobacter spp.			≥ 26	25-22	< 22	≤ 4	> 16
e)	Ampicillin	**2.5 µg**	AMP.L					
	Haemophilus spp.			-	-	< 22	-	> 1
	Penicillin Low	5 µg	PEN.L					
	N. gonorrhoeae			≥ 34	33-23	< 23	≤ 0.06	> 1
	Oxacillin	**1 µg**	OXA.1					
a)	*S. pneumoniae*			≥ 20	-	< 20	≤ 0.06 (pen)	MIC test
d)	*Streptococcus* spp.			≥ 15	-	< 15	≤ 0.12 (pen)	MIC test
l)	*N. gonorrhoeae*			≥ 14	-	< 14	≤ 0.06 (pen)	MIC test
g)	*N. meningitidis*			≥ 12	-	< 12	≤ 0.06 (pen)	MIC test
	Oxacillin	**5 µg**	OXA.5					
a)	*S. pneumoniae*			≥ 24	-	< 24	≤ 0.06 (pen)	MIC test
d)	*Streptococcus* spp.			≥ 20	-	< 20	≤ 0.12 (pen)	MIC test
l)	*N. gonorrhoeae*			≥ 18	-	< 18	≤ 0.06 (pen)	MIC test
g)	*N. meningitidis*			≥ 18	-	< 18	≤ 0.06 (pen)	MIC test
	Amoxycillin	30 µg	AMOXY					
	Haemophilus spp.			-	-	< 30	-	> 1
	Anaerobes (gram pos.)			≥ 24	23-18	< 18	≤ 4	> 16
	Anaerobes (gram neg.)			≥ 32	31-28	< 28	≤ 0.5	> 1
	Amoxycillin+Clav.	30+15 µg	AM+CL					
	Haemophilus spp.			≥ 28	-	-	≤ 4/2	-
	Campylobacter spp.			≥ 28	27-20	< 20	≤ 4/2	> 16/2
	Anaerobes			≥ 28	27-20	< 20	≤ 4/2	> 16/2
	Ticarcillin+Clavulanate	75+15 µg	TI+CL					
	Anaerobes			≥ 24	23-18	< 18	≤ 16/2	> 64/2
	Piperacillin+Tazobactam	100+10µg	PI+TZ					
	Anaerobes			≥ 28	27-20	< 20	≤ 8/4	> 64/4
	Cephalothin	66 µg	CLOTN					
e)	*Haemophilus* spp.			-	-	< 26	-	> 8 (Amp R)
	Ceftizoxime	30 µg	CEZOX					
b)	*S. pneumoniae* (valid for 3rd gen. cephalosporins)			≥ 32	-	< 32	≤ 0.5	> 0.5
	Cefotaxime	30 µg	CFTAX					
	/ Ceftriaxone	30 µg	CETRX					
b)	*S. pneumoniae*			(use Ceftizoxime)				
	N. gonorrhoeae			≥ 40	-	-	≤ 0.25	-
	N. meningitidis			≥ 38	-	-	≤ 0.25	-
	Campylobacter spp.			≥ 24	23-18	< 18	≤ 4	> 32

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	NEO-SENSITABS	POTENCY	CODE	Zone diameter in mm S	I	R	Concentrations critiques S	R
	Cefoxitin	60 µg	CFOXT					
	Anaerobes			-	-	< 22	-	> 32
	Imipenem	15 µg	IMIPM					
	S. pneumoniae			≥ 32	31-28	< 28	≤ 0.5	> 2
	Campylobacter spp.			≥ 24	23-20	< 20	≤ 4	> 8
	Anaerobes			≥ 24	23-20	< 20	≤ 4	> 8
	Meropenem	10 µg	MEROP					
	S. pneumoniae			≥ 32	31-28	< 28	≤ 0.5	> 2
	Ertapenem	10 µg	ERTAP					
	S. pneumoniae			≥ 28	27-24	< 24	≤ 1	≥ 2
	Haemophilus spp.			≥ 28	-	-	≤ 0.5	-
	Anaerobes			≥ 20	19-18	≤ 17	≤ 4	> 8
	Erythromycin	78 µg	ERYTR					
c)	*S. pneumoniae* / *Streptococcus* spp.			≥ 28	27-24	< 24	≤ 1	> 4
	Campylobacter spp.			≥ 28	27-24	< 24	≤ 1	> 4
n)	*Helicobacter pylori*			≥ 28	27-24	< 24	≤ 1	> 4
	Clindamycin	25 µg	CLIND					
	S. pneumoniae / *Streptococcus* spp.			≥ 26	-	< 25	≤ 2	> 2
	Anaerobes			≥ 26	-	< 25	≤ 2	> 2
	Linezolid	30 µg	LINEZ					
	S. pneumoniae / *Streptococcus* spp.			≥ 28	27-25	< 24	≤ 2	> 4
	Telithromycin	15 µg	TEL15					
	S. pneumoniae / *Streptococcus* spp.			≥ 21	20-17	< 17	≤ 0.5	> 2
	Tetracyclines	80 µg	TET80					
	Haemophilus spp.			≥ 30	29-26	< 26	≤ 2	> 4
	S. pneumoniae / *Streptococcus* spp.			≥ 28	27-24	< 24	≥ 4	> 8
	N. gonorrhoeae			-	-	< 26	≤ 1	> 4
	Campylobacter spp.			≥ 28	27-24	< 24	≤ 4	> 8
	Chloramphenicol	60 µg	CLR60					
	Haemophilus spp.			≥ 34	33-30	< 30	≤ 2	> 4
	S. pneumoniae / *Streptococcus* spp.			≥ 30	29-26	< 26	≤ 8	> 16
	N. gonorrhoeae			≥ 32	31-28	< 28	≤ 4	> 16
	N. meningitidis			≥ 34	-	-	≤ 2	> 4
	Campylobacter spp.			≥ 30	29-26	< 26	≤ 8	> 16
	Anaerobes			≥ 30	29-26	< 26	≤ 8	> 16
	Rifampicin	30 µg	RIFAM					
	Haemophilus spp.			≥ 26	25-22	< 22	≤ 2	> 4
	Streptococcus spp.			≥ 22	21-18	< 18	≤ 4	> 16
h)	*N. meningitidis*			≥ 32	-	-	≤ 0.25	-
	Anaerobes			≥ 22	21-18	< 18	≤ 4	> 16
c_2)	Norfloxacin	10 µg	NORFX				Reduced susceptibility to quinolones	
	S. pneumoniae			-	-	< 12	-	> 8
	Ciprofloxacin	10 µg	CIP10					
	Haemophilus spp.			≥ 28	(Use nalidixan)	-	≤ 0.5	-
	N. gonorrhoeae			≥ 36	(Use nalidixan)	< 36	≤ 0.06	> 0.12
	Campylobacter spp.			≥ 28	27-23	< 23	≤ 0.5	> 1
	Helicobacter pylori			≥ 20	-	< 20	≤ 1	> 1
	Ofloxacin	10 µg	OFLOX					
	Haemophilus spp.			≥ 28	(Use nalidixan)	-	≤ 0.5	-
	N. gonorrhoeae			≥ 34	(Use nalidixan)	< 34	≤ 0.12	> 0.25

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	NEO-SENSITABS	POTENCY	CODE	Zone diameter in mm S	I	R	Concentrations critiques S	R
	Levofloxacin	5 µg	LEVOF					
	Haemophilus spp.			≥ 24	(Use nalidixan)	-	≤ 1	-
c2)	*S. pneumoniae*			≥ 18	-	< 18	≤ 2	> 2
	Streptococcus spp.			≥ 20	19-17	≤ 16	≤ 1	> 2
	Gatifloxacin	5 µg	GATIF					
	Haemophilus spp.			≥ 24	(Use nalidixan)	-	≤ 1	-
c2)	*S. pneumoniae* / *Streptococcus* spp.			≥ 21	20-18	< 18	≤ 1	> 2
	Moxifloxacin	5 µg	MOXIF					
	Haemophilus spp.			≥ 28	(Use nalidixan)	-	≤ 0.5	-
c2)	*S. pneumoniae*			≥ 24	-	< 24	≤ 0.5	> 0.5
	Streptococcus spp.			≥ 24	23-21	≤ 20	≤ 0.5	> 1
	Anaerobes			≥ 21	20-18	< 18	≤ 1	> 2
	Nalidixan	130 µg	NALID					
f)	*Haemophilus* spp.			-	-	< 28	decreased susceptibility	
k)	*N. gonorrhoeae*			-	-	< 32	to quinolones	
i)	*N. meningitidis*			-	-	< 28	-	-
	Campylobacter spp.			≥ 28	27-24	< 24	≤ 8	> 16
	Trimethoprim+Sulfa	5.2+240 µg	TR+SU					
	Haemophilus spp.			≥ 36	-	-	≤ 0.5/9.5	> 1/19
	S. pneumoniae / *Streptococcus* spp.			≥ 30	29-26	< 26	≥ 2/38	> 8/152
	Gentamicin	40 µg	GEN40					
	Haemophilus spp.			≥ 26	25-23	< 23	≤ 2	> 4
	Campylobacter spp.			≥ 28	27-23	< 23	≤ 2	> 4
	Tobramycin	40 µg	TOBRA					
	Campylobacter spp.			≥ 28	27-23	< 23	≤ 2	> 4
	Spectinomycin							
	N. gonorrhoeae			≥ 22	-	< 22	≤ 64	> 64
	Teicoplanin	30 µg	TPN30					
	S. pneumoniae/*Streptococcus* spp./anaerobes			≥ 18	-	< 18	≤ 4	> 8
	Vancomycin	5 µg	VAN.5					
	S. pneumoniae / *Streptococcus* spp.			≥ 16	-	< 16	≤ 4	> 8
	Anaerobes			≥ 18	-	< 18	≤ 4	> 8
m)	Metronidazole	16 µg	MTR16					
	Anaerobes			-	-	< 21	≤ 4	> 16
	Clostridium difficile (1)			≥ 32	31-30	< 30	≤ 4	> 16

Remarks:

Pneumococci

a) Oxacillin (1 µg or 5 µg) are used for the detection of reduced sensitivity to penicillin in pneumococci. Penicillin resistant isolates from the meninges must be considered resistant to ampicillin/amoxycillin, amox+clav and first and second generation cephalosporins.

b) Cefotaxime and ceftriaxone must not be tested against pneumococci by the diffusion method. A surrogate test is used instead: Ceftizoxime, ceftizoxime detects reduced sensitivity to third generation cephalosporins. Strains sensitive to ceftizoxime show currently MIC < 0.5 µg/ml towards cefotaxime/ceftriaxone (susceptible), while isolates resistant to ceftizoxime should be tested by an MIC method.

c) Erythromycin: Interpretation valid for azithromycin and clarithromycin.

c_2) Screening of pneumococci for reduced sensitivity to fluoroquinolones is done using Norfloxacin 10 µg Neo-S. If the inhibition zone is < 12 mm (or the MIC is < 16 µg/ml) there is a high risk of development of resistant mutants in vivo.

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Streptococci

Penicillin resistant strains of Group A and Group B streptococci have not yet been recognized.
Viridans streptococci isolated from blood or CSF should be tested for penicillin or ampicillin susceptiblity using an MIC method.

d) Oxacillin (1 µg or 5 µg) are useful for screening for penicillin susceptibility in viridans streptococci.
c) Erythromycin: Interpretation valid for azithromycin and clarithromycin.

H. influenzae

e) Beta-lactamase negative, ampicillin resistant strains (BLNAR) are best detected using Ampicillin 2.5 µg Neo-Sensitabs. Cephalothin Neo-Sensitabs is also useful to detect BLNAR strains (zone < 26 mm). BLNAR isolates must be considered resistant to amoxycillin, amox+clav, as well as first and second generation cephalosporins, no matter the size of the inhibition zone.
f) Strains resistant to nalidixan should be suspected of having reduced susceptibility to quinolones. Strains with reduced susceptibility to ciprofloxacin (MIC ≥ 0.125 µg/ml) show decreased susceptibility to all quinolones.

Meningococci

g) Oxacillin (1 µg or 5 µg) are used routinely for the detection of reduced sensitivity to penicillins, in meningococci (chromosomal resistance).
h) Rifampicin: Used for prophylaxis only (not treatment).
i) Nalidixan is useful to screen for strains with reduced susceptibility to quinolones.

Gonococci

k) Nalidixan is useful to detect strains with reduced susceptibility to quinolones. Ciprafloxacin resistant gonococci should presumably be resistant to all quinolones.
A positive beta-lactamase test predicts resistance to penicillin, amoxycillin/ampicillin, piperacillin and ticarcillin.
l) Oxacillin (1 µg and 5 µg) Neo-Sensitabs are useful to detect betal lactamase negative gonococci with decreased susceptibility to penicillin (chromosomal resistance).

Campylobacter

For *Campylobacter* spp. the absence of zone of inhibition around ß-lactams, aminoglycosides, macrolides or quinolones indicates high level resistance.

Anaerobes

Vancomycin 5 µg, Kanamycin 500 µg and Colistin 10 µg Neo-Sensitabs are very useful for the identification of the most important gram negative bacilli: *B. fragilis* group are resistant to Vancomycin 5 µg, Kanamycin 500 µg and Colistin 10 µg. Prevotella is resistant to Kanamycin 500 µg and Vancomycin 5 µg (zone < 18 mm), while it is variable to Colistin 10 µg. Porphyromonas is sensitive to Vancomycin 5 µg (zone > 18 mm) and resistant to Kanamycin 500 µg and Colistin 10µg. Fusobacterium is sensitive to Kanamycin 500 µg and Colistin 10 µg and resistant to Vancomycin 5 µg.
For species showing slow growth it may be difficult to establish a correlation between MIC's and zone sizes. Use an MIC method.

m) Metronidazole: Certain strains may show false resistance to metronidazole if anaerobiosis is not correct.

Helicobacter pylori

n) Interpretation valid for clarithromycin.

References:

1) Barbut F. et al: Antimicrobial susceptibilities and serogroups of clinical strains of *Clostridium difficile* isolated in France in 1991 and 1997. Antimicr. Ag. Chemother., **43**, 2607-11,1999.
2) Communique 2004 de la Societe Francaise de Microbiologie (CA-SFM).
3) Communique Janvier 2006 de la Societe Francaise de Microbiologie (CA-SFM).

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10.7 VII – Interpretation according to MIC Breakpoints of DIN 58940-4 (Germany)

VII

Rapidly growing bacteria. Interpretation according to the MIC break-points recommended by the German DIN 58940-4 (January 2000) and GENARS (November 2002) Semiconfluent growth (ICS)

		NEO-SENSITABS	POTENCY	CODE	Zone diameter in mm S	I	R	Break-points MIC µg/ml S	R
		Penicillin Low	5 µg	PEN.L					
a)		*Staphylococcus* spp.			≥ 28	-	≤ 27	≤ 0.125	≥ 0.25 (beta-lactamase)
		other			≥ 28	27-17	≤ 16	≤ 0.125	≥ 2
b)		Oxacillin	1 µg	OXA.1					
		S. aureus			≥ 16	15-14	≤ 13	≤ 1	≥ 2
		Coag. neg. staph.			≥ 20	-	≤ 19	≤ 0.25	≥ 0.5
i)	a)	Ampicillin	33 µg	AMP33	≥ 28	27-21	≤ 20	≤ 2	≥ 16
i)	a)	Amoxycillin	30 µg	AMOXY	≥ 28	27-21	≤ 20	≤ 2	≥ 16
a)		Ticarcillin	75 µg	TICAR	≥ 28	27-21	≤ 20	≤ 8	≥ 64
a)		Piperacillin	100 µg	PIPRA	≥ 28	27-21	≤ 20	≤ 4	≥ 64
b)		Ampicillin+Sulbactam	30+30 µg	AM+SU	≥ 28	27-21	≤ 20	≤ 2+8	≥ 16+8
b)		Amoxycillin+Clav.	30+15 µg	AM+CL	≥ 28	27-21	≤ 20	≤ 2+2	≥ 16+2
b)		Ticarcillin+Clavulanate	75+15 µg	TI+CL	≥ 28	27-21	≤ 20	≤ 8+2	≥ 64+2
b)		Piperacillin+Tazobactam	100+10µg	PI+TZ	≥ 28	27-21	≤ 20	≤ 4+4	≥ 64+4
b)		Cefaclor	30 µg	CCLOR	≥ 30	29-24	≤ 23	≤ 1	≥ 8
b)		Cefuroxime (oral)	60 µg	CEFUR	≥ 32	31-24	≤ 23	≤ 1	≥ 8
b)		Cefuroxime (parenteral)	60 µg	CEFUR	≥ 28	27-21	≤ 20	≤ 4	≥ 16
g)	b)	Cefpodoxime	30 µg	CFPOX	≥ 30	29-24	≤ 23	≤ 1	≥ 8
b)		Cefixime	30 µg	CFFIX	≥ 30	29-27	≤ 26	≤ 1	≥ 4
b)		Cephalothin	66 µg	CLOTN	≥ 30	29-21	≤ 20	≤ 2	≥ 16
b)		Cefazolin	60 µg	CFZOL	≥ 28	27-21	≤ 20	≤ 4	≥ 16
b)		Cefoxitin	60 µg	CFOXT	≥ 28	27-21	≤ 20	≤ 4	≥ 16
		S. aureus			≥ 30	29-28	≤ 27	Oxa S	Mec A pos.
		Coag. neg. staph			≥ 34	33-32	≤ 31	Oxa S	Mec A pos.
b)		Ceftazidime	30 µg	CEZDI	≥ 26	25-19	≤ 18	≤ 4	≥ 32
b)		Cefotaxime	30 µg	CFTAX	≥ 28	27-21	≤ 20	≤ 2	≥ 16
b)		Ceftizoxime	30 µg	CEZOX	≥ 28	27-21	≤ 20	≤ 2	≥ 16
b)		Ceftriaxone	30 µg	CETRX	≥ 26	25-19	≤ 18	≤ 4	≥ 32
b)		Cefepime	30 µg	CFEPM	≥ 26	25-19	≤ 18	≤ 4	≥ 32
b)		Cefotetan	30 µg	CFTTN	≥ 26	25-19	≤ 18	≤ 4	≥ 32
b)		Aztreonam	30 µg	AZTRM	≥ 28	27-19	≤ 18	≤ 2	≥ 32
l)	b)	Meropenem	10 µg	MEROP	≥ 26	25-19	≤ 18	≤ 2	≥ 8
		Ps. aeruginosa			≥ 30	29-24	≤ 23	≤ 2	≥ 8
l)	b)	Imipenem	15 µg	IMIPM	≥ 26	25-21	≤ 20	≤ 2	≥ 8
		Ps. aeruginosa			≥ 30	29-24	≤ 23	≤ 2	≥ 8
j)		Tetracyclines	80 µg	TET80	≥ 28	27-21	≤ 20	≤ 1	≥ 8
		Doxycycline	80 µg	DOXYC	≥ 28	27-21	≤ 20	≤ 1	≥ 8
		Chloramphenicol	60 µg	CLR60	≥ 26	-	≤ 25	≤ 8	≥ 16

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	NEO-SENSITABS	POTENCY	CODE	Zone diameter in mm S	I	R	Break-points MIC µg/ml S	R
c)	Gentamicin	**40 µg**	GEN40	≥ 26	25-21	≤ 20	≤ 1	≥ 8
e)	Gentamicin	**250 µg**	GN250	≥ 15	-	< 14 (HLR)	-	> 500
	Tobramycin	40 µg	TOBRA	≥ 26	25-21	≤ 20	≤ 1	≥ 8
	Netilmicin	40 µg	NETIL	≥ 26	25-21	≤ 20	≤ 1	≥ 8
	Amikacin	40 µg	AMIKA	≥ 24	23-19	≤ 18	≤ 4	≥ 32
e)	Streptomycin	500 µg	ST500	≥ 15	-	< 14 (HLR)	-	> 1000
*	Kanamycin	100 µg	KANAM					
m)	*Staphylococcus* spp.			≥ 23	22-19	≤ 18	≤ 16	≥ 64
f) k)	Vancomycin	**5 µg**	VAN.5	≥ 16	-	≤ 15	≤ 4	≥ 16
k)	Teicoplanin	30 µg	TPN30	≥ 16	-	≤ 15	≤ 4	≥ 16
	Fucidin	100 µg	FUCID	≥ 30	-	≤ 29	≤ 2	≥ 4
	Erythromycin	78 µg	ERYTR	≥ 28	27-24	≤ 23	≤ 1	≥ 8
	Azithromycin	30 µg	AZITR	≥ 20	19-15	≤ 14	≤ 2	≥ 8
	Clindamycin	25 µg	CLIND	≥ 28	27-24	≤ 23	≤ 1	≥ 8
	Telithromycin	15 µg	TEL15	≥ 21	20-17	≤ 16	≤ 0.5	≥ 2
	Linezolid	15 µg	LINEZ	≥ 28	27-24	≤ 23	≤ 2	≥ 8
	Quinupristin/Dalfopristin	15 µg	SYN15	≥ 22	21-18	≤ 17	≤ 1	≥ 4
	Ofloxacin	10 µg	OFLOX	≥ 24	23-19	≤ 18	≤ 1	≥ 4
	Ciprofloxacin	10 µg	CIP10	≥ 24	23-19	≤ 18	≤ 1	≥ 4
	Norfloxacin	10 µg	NORFX	≥ 24	23-19	≤ 18	≤ 1	≥ 4
	Sparfloxacin	10 µg	SPARF	≥ 24	23-19	≤ 18	≤ 1	≥ 4
	Gatifloxacin	5 µg	GATIF	≥ 26	25-23	≤ 22	≤ 1	≥ 4
	Levofloxacin	5 µg	LEVOF	≥ 26	25-23	≤ 22	≤ 1	≥ 4
	Moxifloxacin	5 µg	MOXIF	≥ 26	25-23	≤ 22	≤ 1	≥ 4
d)	Nalidixan	130 µg	NALID	≥ 28	27-24	≤ 23	≤ 8	≥ 32
	Trimethoprim	5.2 µg	TRIME	≥ 20	19-17	≤ 16	≤ 2	≥ 8
	Trim+Sulfa	5.2+240 µg	TR+SU	≥ 28	27-24	≤ 23	≤ 16	≥ 128
	Polymyxins (colistin)	150 µg	CO150	≥ 22	-	≤ 21	≤ 0.5	≥ 4
	Pipemidic acid	30 µg	PIPEM	≥ 22	-	≤ 21	≤ 4	≥ 8
h)	Nitrofurantoin	260 µg	NITRO	≥ 24	-	≤ 23	≤ 64	≥ 512
	Fosfomycin	70+40 µg	FOSFO	≥ 24	23-21	≤ 20	≤ 32	≥ 64
	Mupirocin	10 µg	MUPIR	≥ 18	-	≤ 17	≤ 4	≥ 8
	Rifampicin	30 µg	RIFAM	≥ 26	25-23	≤ 22	≤ 1	≥ 4

Remarks:

Note. This table is valid for rapidly growing bacteria. Fastidious strains and problem organisms have special interpretation tables (haemophilus, neisseria, pneumococci, streptococci etc.) or need special methodologies. See chapter 15 in the User's Guide.

a) **Staphylococci** resistant to Penicillin Low (beta-lactamase producers) should be reported as resistant to penicillin, amoxycillin, ampicillin, azlocillin, piperacillin and ticarcillin.

b) **Oxacillin resistant staphylococci** should be reported as resistant to all other beta-lactams: penicillins, beta-lactamase inhibitor combinations, cephalosporins and carbapenems.

c) **Staphylococci resistant to gentamicin**, should be reported as resistant to all aminoglycosides, except streptomycin.

d) ***E. coli*, Klebsiella and Salmonella** strains resistant to Nalidixan Neo-Sensitabs (zone < 28 mm) show a decreased susceptibility to quinolones, (CIPRO MIC ≥ 0.125 µg/ml). It may result in treatment failure with quinolones.

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e) When testing **enterococci** use Gentamicin 250 µg and Streptomycin 500 µg to detect high level resistance (HLR). Strains that are HLR to gentamicin should be reported HLR to all aminoglycosides (except streptomycin).

f) When testing **enterococci** use Vancomycin 5 µg Neo-Sensitabs. Plates should be incubated for **full 24 hours** and examined carefully for the presence of a haze or other growth within the zone (resistance).

g) Strains of ***E. coli*** and ***Klebsiella*** spp. that produce **ESBL**, may be clinically resistant to therapy with penicillins, cephalosporins or aztreonam, despite apparent in vitro susceptibility. See chapter 16.1 for ESBL screening and confirmatory tests. Strains showing zone ≤ 23 mm with Cefpodoxime Neo-Sensitabs should be suspected of ESBL.

h) Klebsiella, Enterobacter and Proteus should be reported **R** to nitrofurantoin.

i) Klebsiella end Enterobacter should be reported **R** to ampicillin/amoxycillin.

j) *Ps. aeruginosa, St. maltophilia* and *Proteus* spp. should be reported **R** to tetracyclines.

k) Concerning detection of VISA/GISA strains, see User's Guide, chapters 13.1 and 13.5.

m) Interpretation valid for amikacin and isepamicin with staphylococci.

References:

1) "Methoden zur Empfindlichkeitsprüfung von mikrobiellen Krankheitserregern gegen Chemotherapeutika" DIN 58940-4 Bbl. 1, Jan. 2000.
2) GENARS: German Network for Antimicrobial Resistance Surveillance, November 2002.

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10.8 VIII – Interpretation according to the MIC break-points recommended by EUCAST

VIII

Interpretation according to the MIC break-points recommended by the EUCAST
Media: Iso-sensitest, Inoculum: Semiconfluent growth (ICS)

NEO-SENSITABS	POTENCY	CODE	Zone diameter in mm S	I	R	Break-points MIC µg/ml S	R
1) Aminoglycosides:							
Amikacin	40 µg	AMIKA	≥ 24	23-20	< 20	≤ 8	> 16
Coag. neg. staph.			≥ 30	29-26	< 26		
Gentamicin	40 µg	GEN40					
Enterobacteriaceae			≥ 26	25-22	< 22	≤ 2	> 4
Pseudomonas spp./*Acinetobacter* spp.			≥ 28	27-24	< 24	≤ 4	> 4
S. aureus			≥ 30	29-26	< 26	≤ 1	> 1
Coag. neg. staph.			≥ 34	-	< 34	≤ 1	> 1
Netilmicin	40 µg	NETIL					
Enterobacteriaceae			≥ 26	25-22	< 22	≤ 2	> 4
Pseudomonas spp./*Acinetobacter* spp.			≥ 28	27-24	< 24	≤ 4	> 4
S. aureus			≥ 30	29-26	< 26	≤ 1	> 1
Coag. neg. staph.			≥ 34	-	< 34	≤ 1	> 1
Tobramycin	40 µg	TOBRA					
Enterobacteriaceae			≥ 26	25-22	< 22	≤ 2	> 4
Pseudomonas spp./*Acinetobacter* spp.			≥ 28	27-24	< 24	≤ 4	> 4
S. aureus			≥ 30	29-26	< 26	≤ 1	> 1
Coag. neg. staph.			≥ 34	-	< 34	≤ 1	> 1
2) Quinolones:							
Nalidixan	130 µg	NALID					
Enterobacteriaceae			-	-	< 28	} Reduced susceptibility	
Haemophilus spp.			-	-	< 30	} to quinolones	
Ciprofloxacin	10 µg	CIP10					
Enterobacteriaceae (nal)			≥ 28	27-24	< 24	≤ 0.5	> 1
Pseudomonas spp.			≥ 30	29-27	< 27	≤ 0.5	> 1
Acinetobacter spp./Staphylococci			≥ 26	25-22	< 22	≤ 1	> 1
S. pneumoniae			≥ 36	35-22	< 22	≤ 0.12	> 2
H. influenzae/Moraxella spp. (nal)			≥ 30	29-26	< 26	≤ 0.5	> 0.5
Gonococci/Meningococci (nal)			≥ 36	-	< 36	≤ 0.03	> 0.06
Levofloxacin	5 µg	LEVOF					
Enterobacteriaceae			≥ 26	25-23	< 23	≤ 1	> 2
Pseudomonas spp.			≥ 28	27-24	< 24	≤ 1	> 2
Streptococcus spp.			≥ 22	21-19	< 19	≤ 1	> 2
S. pneumoniae			≥ 22	21-19	< 19	≤ 2	> 2
H. influenzae/Moraxella spp. (nal)			≥ 28	27-24	< 24	≤ 1	> 1
Moxifloxacin	5 µg	MOXIF					
Enterobacteriaceae (nal)			≥ 28	27-24	< 24	≤ 0.5	> 1
Staphylococci			≥ 28	27-24	< 24	≤ 0.5	> 1
S. pneumoniae			≥ 24	23-22	< 22	≤ 0.5	> 0.5
H. influenzae/Moraxella spp. (nal)			≥ 30	29-26	< 26	≤ 0.5	> 0.5
Norfloxacin	10 µg	NORFX					
Enterobacteriaceae (nal)			≥ 28	27-24	< 24	≤ 0.5	> 1

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NEO-SENSITABS	POTENCY	CODE	Zone diameter in mm S	I	R	Break-points MIC µg/ml S	R
Ofloxacin	10 µg	OFLOX					
Enterobacteriaceae (nal)			≥ 28	27-24	< 24	≤ 0.5	> 1
Staphylococci			≥ 26	25-22	< 22	≤ 1	> 1
S. pneumoniae			≥ 36	35-20	< 20	≤ 0.12	> 4
H. influenzae/Moraxella spp. (nal)			≥ 30	29-26	< 26	≤ 0.5	> 0.5
Gonococci (nal)			≥ 34	-	< 34	≤ 0.12	> 0.25
3) Glycopeptides:							
Teicoplanin	30 µg	TPN30					
** Staphylococci/Enterococci			≥ 16	15-14	< 14	≤ 4	> 8
Streptococci/*S. pneumoniae*			≥ 18	17-16	< 16	≤ 4	> 4
Vancomycin	5 µg	VAN.5					
** Staphylococci/Enterococci			≥ 16	15-14	< 14	≤ 4	> 8
Streptococci/*S. pneumoniae*			≥ 18	17-16	< 16	≤ 4	> 4
4) Oxazolidinones:							
Linezolid	30 µg	LINEZ					
Staphylococci/Enterococci			≥ 26	25-22	< 22	≤ 4	> 4
Streptococci/*S. pneumoniae*			≥ 28	27-23	< 23	≤ 2	> 4
5) Cephalosporins:							
Cefepime	30 µg	CFEPM				≤ 4	> 8
Enterobacteriaceae			≥ 30	29-24	< 24	≤ 1	> 8
Pseudomonas spp.			≥ 28	-	< 28	≤ 8	> 8
S. pneumoniae				Test ceftizoxime		≤ 1	> 2
Streptococcus spp.			≥ 32	31-28	< 28	≤ 0.5	> 0.5
H. influenzae/*Moraxella catarrhalis*			≥ 35	34-31	< 31	≤ 0.25	> 0.25
Cefotaxime	30 µg	CFTAX				≤ 1	> 2
Enterobacteriaceae			≥ 30	29-24	< 24	≤ 1	> 2
S. pneumoniae				Test ceftizoxime		≤ 0.5	> 2
Streptococcus spp.			≥ 32	31-28	< 28	≤ 0.5	> 0.5
H. influenzae/Moraxella catarrhalis			≥ 38	37-34	< 34	≤ 0.12	> 0.12
N. meningitidis			≥ 38	37-34	< 34	≤ 0.12	> 0.12
N. gonorrhoeae			≥ 38	37-34	< 34	≤ 0.12	> 0.12
Ceftazidime	30 µg	CEZDI				≤ 4	> 8
Enterobacteriaceae			≥ 30	29-24	< 24	≤ 1	> 8
Pseudomonas spp.			≥ 28	-	< 28	≤ 8	> 8
Ceftriaxone	30 µg	CETRX				≤ 1	> 2
Enterobacteriaceae			≥ 30	29-24	< 24	≤ 1	> 2
S. pneumoniae				Test ceftizoxime		≤ 0.5	> 2
Streptococcus spp.			≥ 32	31-28	< 28	≤ 0.5	> 0.5
H. influenzae/Moraxella catarrhalis			≥ 36	35-32	< 32	≤ 0.12	> 0.12
N. meningitidis			≥ 36	35-32	< 32	≤ 0.12	> 0.12
N. gonorrhoeae			≥ 36	35-32	< 32	≤ 0.12	> 0.12
Cefuroxime	60 µg	CEFUR				≤ 4	> 8
Enterobacteriaceae			≥ 26	-	< 26	≤ 8	> 8
S. pneumoniae (non-meningeal)			≥ 36	35-32	< 32	≤ 0.5	> 1
Streptococcus spp.			≥ 36	35-34	< 34	≤ 0.5	> 0.5
H. influenzae/Moraxella catarrhalis			≥ 36	35-30	< 30	≤ 1	> 2
Ceftizoxime	30 µg	CEZOX					
S. pneumoniae (3rd gen. cephalosporins)			≥ 32	31-28	< 28	≤ 0.5	MIC

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NEO-SENSITABS	POTENCY	CODE	Zone diameter in mm S	I	R	Break-points MIC µg/ml S	R
6) Carbapenems:							
Ertapenem	10 µg	ETP10				≤ 0.5	> 1
Enterobacteriaceae			≥ 30	29-26	< 26	≤ 0.5	> 1
Streptococci/*S. pneumoniae*			≥ 30	29-26	< 26	≤ 0.5	> 0.5
H. influenzae/Moraxella catarrhalis			≥ 30	29-26	< 26	≤ 0.5	> 0.5
Anaerobes			≥ 32	31-28	< 28	≤ 1	> 1
Imipenem	15 µg	IMIPM				≤ 2	> 8
Enterobacteriaceae			≥ 26	25-20	< 20	≤ 2	> 8
Pseudomonas spp.			≥ 28	27-24	< 24	≤ 4	> 8
Acinetobacter			≥ 26	23-18	< 18	≤ 2	> 8
Streptococci/*S. pneumoniae*			≥ 28	27-25	< 25	≤ 2	> 2
Enterococcus spp.			≥ 24	23-20	< 20	≤ 4	> 8
H. influenzae/Moraxella catarrhalis			≥ 32	31-28	< 28	≤ 2	> 2
Anaerobes			≥ 28	27-20	< 20	≤ 2	> 8
Meropenem	10 µg	MEROP					
Enterobacteriaceae			≥ 26	25-20	< 20	≤ 2	> 8
Pseudomonas spp.			≥ 30	29-24	< 24	≤ 2	> 8
Acinetobacter			≥ 26	25-20	< 20	≤ 2	> 8
Streptococci/*S. pneumoniae*			≥ 28	27-25	< 25	≤ 2	> 8
H. influenzae/Moraxella catarrhalis			≥ 28	27-24	< 24	≤ 2	> 2
N. meningitidis			≥ 32	31-28	< 28	≤ 0.25	> 0.25
Anaerobes			≥ 32	31-26	< 26	≤ 2	> 8
7) Others:							
Aztreonam	30 µg	AZTRM				≤ 4	> 8
Enterobacteriaceae			≥ 30	29-24	< 24	≤ 1	> 8
Pseudomonas spp.			≥ 32	31-20	< 20	≤ 1	> 16
Tigecycline	15 µg	TIG15				≤ 0.25	> 0.5
Enterobacteriaceae			≥ 24	23-20	< 20	≤ 1	> 2
Staphylococci			≥ 26	-	< 26	≤ 0.5	> 0.5
Streptococcus spp. (non *S. pneumoniae*)			≥ 25	24-20	< 20	≤ 0.25	> 0.5
Enterococcus spp.			≥ 21	-	< 21	≤ 0.25	> 0.5
S. pnemoniae			≥ 24	-	< 24	≤ 0.25	> 0.5
Haemophilus spp.			≥ 28	-	< 28	≤ 0.25	> 0.25
Daptomycin	30 µg	DAPCa					
Prediffusion 2+18 h. *							
Staphylococci			≥ 22	21-20	< 20	≤ 1	> 1
Streptococcus spp. (non *S. pneumoniae)*			≥ 22	21-20	< 20	≤ 1	> 1
Enterococci			≥ 12	-	< 12	≤ 4	> 4

nal = Use Nalidixan Neo-Sensitabs as surrogate test. Strains resistant to Nalidixan show reduced susceptibility to fluoroquinolones.

5) 6) 7) Zones tentative for 1 year.

* Technique described on page 18.

** Prediffusion technique for detecting GISA/VISA strains as well as VRE described on page 18.

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11 Interpretation of Results

Susceptible (S):
The infection due to the strain tested may be expected to respond to a normal dosage of this antimicrobial.

Intermediate (I):
The intermediate category implies clinical applicability in body sites where the drugs are concentrated (e.g. urine) or when high dosage of an antimicrobial can be used (e.g. betalactams).

The intermediate category also comprises a "buffer zone" which should prevent small uncontrolled technical factors from causing major discrepancies in interpretations; thus, when a zone falls within the intermediate range, the results may be considered equivocal, and if alternative drugs are not available MIC testing may be indicated.

Resistant (R):
The antimicrobial cannot be recommended for treatment in this case.

If only “S” criteria are specified:
For some organism/antimicrobial combinations, the absence of resistant strains precludes defining any category other than susceptible. For strains yielding results suggestive of "non susceptible", organism identification and antimicrobial susceptibility test results should be confirmed. Subsequently the isolates should be submitted to a Reference Laboratory for further testing.

References:
1) CLSI: Performance Standards for Antimicrobial Disk Susceptibility Testing. 8th ed. **M2-A8**, 2003.

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12 Quality Control Procedures

The goals of a good Quality Control Program are:

• To control precision and accuracy of the test procedure.

• To control performance of the reagents used in the test.

• To control the performance of the staff who carry out the tests and read the results.

Stock cultures of *Staphylococcus aureus* ATCC 25923, *E. coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, and *Enterococcus faecalis* ATCC 29212 should be obtained from a reliable source. Quality control stock organisms may be obtained from the ROSCO representative in your country. Other strains recommended as quality control strains are *Streptococcus pneumoniae* ATCC 49619 (see chapter 15.5.1), *Haemophilus influenzae* ATCC 49247 (see chapter 15.1.1), *E. faecalis* ATCC 51299 (see chapter 14.5), *S. aureus* ATCC 43300 (see chapter 13.4), and *S. aureus* 700788 (see chapter 13.5).

Store working control cultures on Tryptic soy agar at 4-8°C and subculture weekly. Replace working cultures once a month from frozen, liophylized or commercial cultures.

Before testing, the strains should be streaked onto agar plates to obtain single colonies. Colonies should then be picked and suspended in 0.9 % saline, for testing according to the recommended susceptibility testing procedures.

Each laboratory should record the result of consecutive separate analysis using the control cultures and each antimicrobial to be controlled. If an unexplained result suggests a change in the organism's susceptibility, a fresh culture of the control strain should be obtained.

Zone Diameters Quality Control Limits

Quality control should be performed at least once a week and every time a new lot of Mueller-Hinton is introduced.

The control limits, using Mueller-Hinton Agar and inoculum according to CLSI (Kirby-Bauer), have been established as follows:

Control limits on Mueller-Hinton agar Inoculum according to CLSI (Kirby-Bauer)

			Zone diameter in mm			
NEO-SENSITABS		**CODE**	**E. coli ATCC 25922**	**S. aureus ATCC 25923**	**Ps. aeruginosa ATCC 27853**	**Ent. faecalis ATCC 29212**
AMIKACIN	40 µg	AMIKA	24-30	22-29	25-31	-
AMOXYCILLIN	30 µg	AMOXY	21-26	-	-	26-32
AMOXYCILLIN+ CLAVULANATE	30+15 µg	AM+CL	22-27	28-36	-	-
AMPICILLIN	33 µg	AMP33	22-27	-	-	25-31
AZITHROMYCIN	30 µg	AZITR	-	21-27	-	-
AZTREONAM	30 µg	AZTRM	29-36	-	24-30	-
CEFTOBIPROLE	30 µg	CFBIP	30-36	26-34	23-31	-
CARBENICILLIN	115 µg	CARBE	26-32	-	21-27	-
CEFACLOR	30 µg	CCLOR	22-27	25-33	-	-
CEFAZOLIN	60 µg	CFZOL	26-32	28-36	-	-
CEFEPIME	30 µg	CFEPM	31-38	-	26-33	-
CEFPIROME	30 µg	CFPIR	31-38	-	26-32	18-22
CEFOTAXIME	30 µg	CFTAX	30-36	28-34	18-25	-
CEFOTETAN	30 µg	CFTTN	30-36	15-20	-	-
CEFOXITIN	60 µg	CFOXT	29-35	26-34	-	-

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NEO-SENSITABS		CODE	Zone diameter in mm E. coli ATCC 25922	S. aureus ATCC 25923	Ps. aeruginosa ATCC 27853	Ent. faecalis ATCC 29212
CEFSULODIN	30 µg	CFSUL	-	-	27-33	-
CEFTAZIDIME	30 µg	CEZDI	27-33	18-23	22-30	-
CEFTRIAXONE	30 µg	CETRX	29-35	21-28	17-24	-
CEFUROXIME	60 µg	CEFUR	24-30	29-36	-	-
CEPHALEXIN	30 µg	CFLEX	16-21	-	-	-
CEPHALOTHIN (Cephalosporins)	66 µg	CLOTN	21-27	32-40	-	18-23
CHLORAMPHENICOL (clear zone)	60 µg	CLR60	24-32	23-30	-	21-27
CINOXACIN	30 µg	CINOX	20-26	-	-	-
CIPROFLOXACIN	**10 µg**	CIP10	30-40	21-29	24-32	17-24
CIPROFLOXACIN	**0.5 µg**	CIP.L	24-34	-	-	-
CLARITHROMYCIN	30 µg	CLARI	-	24-31	-	-
CLINDAMYCIN	25 µg	CLIND	-	30-38	-	-
COLISTIN (2+18 h. predif.)	10 µg	Co.10	22-28	-	17-23	-
DAPTOMYCIN (2+18 h. prediffusion)	30 µg	DAPCa	-	22-28	-	-
DOXYCYCLINE	80 µg	DOXYC	25-31	29-35	-	18-23
DORIPENEM	10 µg	DORIP	28-35	33-42	29-35	-
ERTAPENEM	10 µg	ERTAP	29-36	24-31	13-21	-
ERYTHROMYCIN	78 µg	ERYTR	-	26-33	-	19-24
FAROPENEM	10 µg	FAROP	20-26	27-24	-	-
FOSFOMYCIN	70+40 µg	FOSFO	25-33	-	28-35	-
FUCIDIN	100 µg	FUCID	-	28-38	-	-
GATIFLOXACIN	5 µg	GATIF	30-37	27-33	20-28	
GENTAMICIN	**40 µg**	GEN40	25-31	25-32	25-31	-
GENTAMICIN	**250 µg**	GN250	-	-	-	17-23
IMIPENEM	15 µg	IMIPM	29-34	-	23-31	26-31
KANAMYCIN	100 µg	KANAM	26-32	25-31	-	-
LEVOFLOXACIN	5 µg	LEVOF	30-38	26-31	21-28	-
LINEZOLID	30 µg	LINEZ	-	25-31	-	21-25
MECILLINAM	33 µg	MECIL	27-35	-	-	-
MEROPENEM	10 µg	MEROP	31-38	32-42	31-38	18-24
MINOCYCLINE	80 µg	MINOC	24-30	27-33	-	-
MOXIFLOXACIN	5 µg	MOXIF	28-35	28-35	17-25	-
MUPIROCIN	10 µg	MUPIR	-	21-26	-	-
NALIDIXAN	130 µg	NALID	27-33	-	-	-
NEOMYCIN	120 µg	NEOMY	23-28	23-30	-	-
NETILMICIN	40 µg	NETIL	24-30	25-32	22-27	-
NITROFURANTOIN	260 µg	NITRO	24-30	24-30	-	24-29
NORFLOXACIN	10 µg	NORFX	28-35	17-26	19-26	12-17
NOVOBIOCIN	5 µg	NOV.5	-	18-25	-	-
OFLOXACIN	10 µg	OFLOX	29-38	23-29	22-26	16-22
OXACILLIN	**1 µg**	OXA.1	-	18-24	-	-
OXACILLIN	**5 µg**	OXA.5	-	22-28	-	-
OXOLINIC ACID	10 µg	OXOLI	30-36	18-24	-	-

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NEO-SENSITABS		CODE	Zone diameter in mm E. coli ATCC 25922	S. aureus ATCC 25923	Ps. aeruginosa ATCC 27853	Ent. faecalis ATCC 29212
PENICILLIN LOW	5 µg	PEN.L	-	26-36	-	15-21
PIPEMIDIC ACID	30 µg	PIPEM	26-32	-	-	-
PIPERACILLIN	100 µg	PIPRA	26-31	-	25-33	25-30
PIPERACILLIN+ TAZOBACTAM	100+10 µg	PI+TZ	26-31	-	25-33	-
POLYMYXINS	150 µg	CO150	19-24	-	20-25	-
QUINUPRISTIN/ DALFOPRISTIN	15 µg	SYN15	-	21-28	-	-
RIFAMPICIN	30 µg	RIFAM	-	32-40	-	-
SULPHONAMIDES	240 µg	SULFA	18-25	23-33	-	-
TEICOPLANIN	30 µg	TPN30	-	15-21	-	-
TELAVANCIN	30 µg	TELAV	-	16-20	-	-
TELITHROMYCIN	15 µg	TEL15	-	24-30	-	-
TEMOCILLIN	30 µg	TEMOC	18-24	-	-	-
TETRACYCLINES	80 µg	TET80	26-32	26-34	-	15-21
TICARCILLIN	75 µg	TICAR	24-30	-	22-28	-
TICARCILLIN+ CLAVULANATE	75+15 µg	TI+CL	25-31	-	24-30	-
TIGECYCLINE	15 µg	TIG15	20-27	20-25	9-13	-
TOBRAMYCIN	40 µg	TOBRA	25-30	26-32	28-34	-
TRIMETHOPRIM	5.2 µg	TRIME	22-29	19-25	-	21-27
TRIMETHOPRIM+ SULFA	5.2+240 µg	TR+SU	30-38	29-38	-	26-32
VANCOMYCIN	5 µg	VAN.5	-	16-21	-	15-18

NEO-SENSITABS		CODE	Zone diameter in mm E. coli ATCC 35218
AMOXYCILLIN+CLAVULANATE	30+15 µg	AM+CL	21-26
AMPICILLIN+SULBACTAM	30+30 µg	AM+SU	21-26
PIPERACILLIN+TAZOBACTAM	100+10 µg	PI+TZ	26-31
TICARCILLIN+CLAVULANATE	75+15 µg	TI+CL	25-31
TEMOCILLIN	30 µg	TEMOC	20-26

Frequency of Q.C. Testing according to CLSI

1) **Daily Testing**. When testing is performed daily, for each antimicrobial/organism combination, 1 out of 20 consecutive results may be out of the acceptable range. More than 1 out of control result in 20 consecutive tests requires corrective action.

2) **Weekly Testing**. Satisfactory performance should be demonstrated for conversion from daily to weekly Q.C. testing.
   - Test all control strains for 20 consecutive days.
   - No more than 3 out of the 30 zone diameters for each antimicrobial/organism combination may be out of the acceptable range.
   - If any of the weekly quality control results is out of the acceptable range, corrective action is required.

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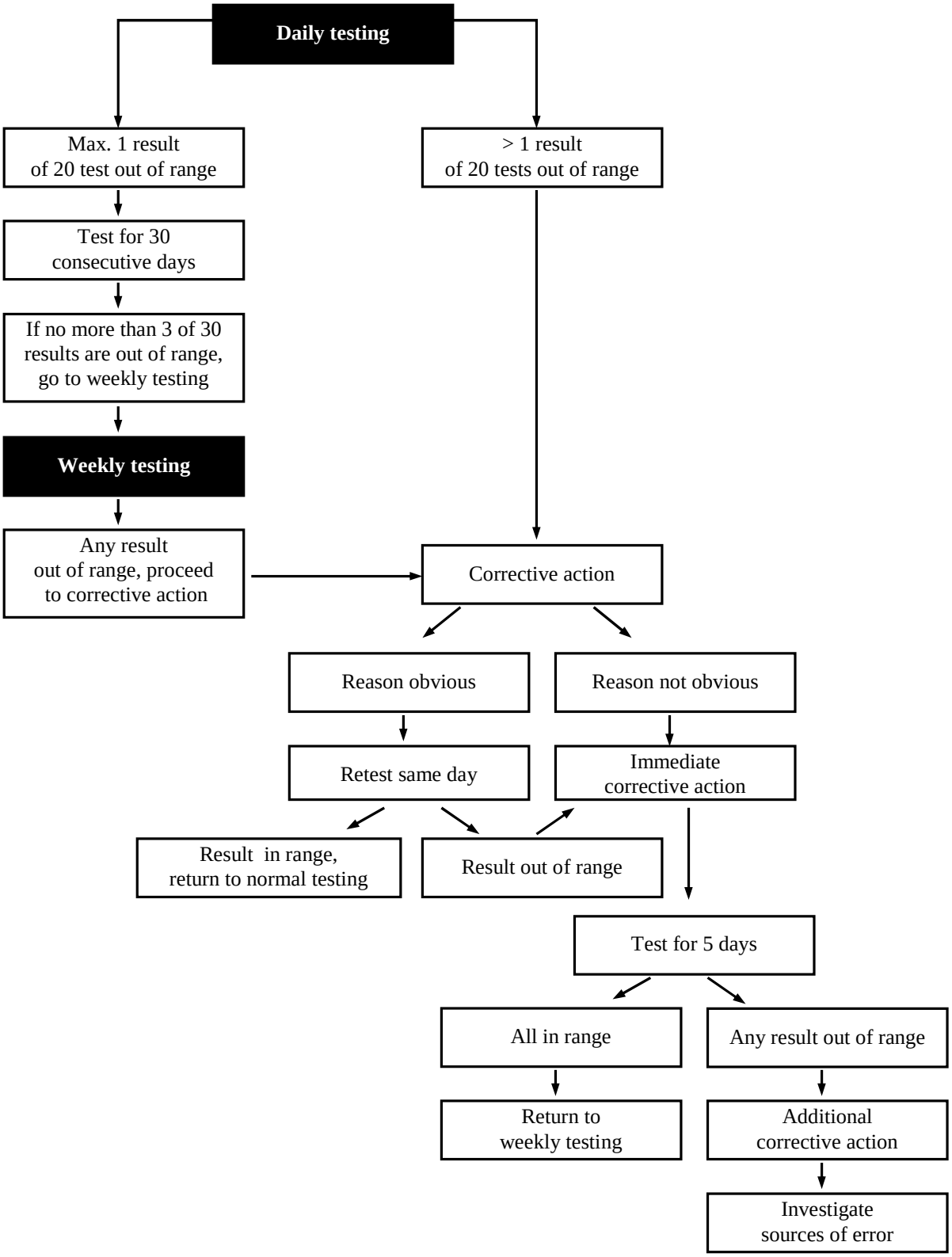
12.1 Quality Control Flow Chart


Daily testing
Max. 1 result of 20 test out of range
> 1 result of 20 tests out of range
Test for 30 consecutive days
If no more than 3 of 30 results are out of range, go to weekly testing
Weekly testing
Any result out of range, proceed to corrective action
Corrective action
Reason obvious
Reason not obvious
Retest same day
Immediate corrective action
Result in range, return to normal testing
Result out of range
Test for 5 days
All in range
Any result out of range
Return to weekly testing
Additional corrective action
Investigate sources of error

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12.2 Quality Control Zones on Danish Blood Agar

Kontrolgrænser på DBA (SSI-substrat)
Inokulum: Semi-konfluerende vækst

			Zone diameter i mm			
NEO-SENSITABS		**CODE**	**E. coli ATCC 25922**	**S. aureus ATCC 25923**	**Ps. aeruginosa ATCC 27853**	**Ent. faecalis ATCC 29212**
Amoxycillin+Clavulanate	30+15 µg	AM+CL	25-30	-	-	30-37
Ampicillin	33 µg	AMP33	26-32	-	-	30-38
Cefotaxime	30 µg	CFTAX	38-43	-	-	-
Cefoxitin	**10 µg**	CFO10		22-26		
Cefoxitin	**60 µg**	CFOXT		30-36		
Ceftazidime	30 µg	CEZDI	35-40	-	32-38	-
Ceftriaxone	30 µg	CETRX	37-42	-	-	-
Cefuroxime	60 µg	CEFUR	28-34	-	-	-
Cephalothin	66 µg	CLOTN	24-28	-	-	-
Chloramphenicol	60 µg	CLR60	31-35	28-34	-	26-31
Ciprofloxacin	10 µg	CIP10	36-44	-	33-39	24-29
Clindamycin	25 µg	CLIND	-	34-39	-	-
Erythromycin	78 µg	ERYTR	-	31-37	-	-
Fucidin	100 µg	FUCID	-	26-32	-	-
Gentamicin	40 µg	GEN40	28-34	31-36	30-35	18-24
Imipenem	15 µg	IMIPM	35-41	-	30-36	-
Mecillinam	33 µg	MECIL	34-40	-	-	-
Methicillin	29 µg	METHI	-	30-37	-	-
Nalidixan	130 µg	NALID	28-33	-	-	-
Netilmicin	40 µg	NETIL	29-34	33-37	28-33	-
Nitrofurantoin	260 µg	NITRO	26-32	26-31	-	33-38
Ofloxacin	10 µg	OFLOX	33-38	-	27-33	25-29
Oxacillin	1 µg	OXA.1	-	21-27	-	-
Penicillin Low	5 µg	PEN.L	-	34-40	-	15-19
Piperacillin	100 µg	PIPRA	30-36	-	34-40	31-36
Sulphonamides	240 µg	SULFA	25-32	-	-	-
Teicoplanin	60 µg	TEICO	-	20-24	-	-
Tetracyclines	80 µg	TET80	33-38	34-39	-	22-27
Ticarcillin	75 µg	TICAR	28-34	-	28-33	-
Tobramycin	40 µg	TOBRA	27-31	31-36	31-35	-
Trimethoprim	5.2 µg	TRIME	26-33	22-26	-	39-43
Trimethoprim+Sulfa	5.2+240 µg	TR+SU	38-43	35-41	-	42-47
Vancomycin	5 µg	VAN.5	-	17-22	-	16-20

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13 Detection of Resistant Staphylococci Against Methicillin and Vancomycin

13.1 *Staphylococcus aureus*

a) Methicillin resistant *Staphylococcus aureus* (MRSA) and *S. lugdunensis*

Methicillin-resistant staphylococci are one of the leading causes of nosocomial infections world-wide. Most methicillin resistance is mediated by 1) the mecA-gene determinant, but a resistant phenotype can also be achieved by 2) hyper-production of beta-lactamase and 3) the presence of PBP's with decreased affinity for beta-lactams (PBP 2a or PBP 2') (1).

For susceptibility testing of *S. aureus* and *S. lugdunensis* we recommend:
- **Cefoxitin** Neo-Sensitabs
- Oxacillin 1 µg Neo-Sensitabs (only *S. aureus*)
- Mueller-Hinton (no NaCl added)
- Inoculum from direct colony suspension (McFarland 0.5 standard)
- Incubate for full 24 hours at 33-35 °C.
- Interpretation: S ≥ 25 mm, R ≤ 24 mm with Cefoxitin 60 µg Neo-Sensitabs.
  S ≥ 13 mm, I: 12-11 mm, R: ≤ 10 mm with Oxacillin 1 µg Neo-Sensitabs.
  In case of discrepancy (Oxa S and Cefox R), the cefoxitin result should be used.

The cefoxitin test is the preferred method for testing *S. aureus, S. lugdunensis* and coagulase negative staphylococci for resistance to the penicillinase-stable penicillins (CLSI 2005) (43).

According to Felten (14) the cefoxitin diffusion method is the best performing test for routine detection of all classes of MRSA. According to the Working Group on staphylococci (NCCLS/CLSI 2005) the results of disk/tablet diffusion using cefoxitin can be used to predict mecA-mediated resistance in stapylococci. Cefoxitin Neo-Sensitabs should be used to detect MRSA (heterogeneous resistance).

Isolates of staphylococci that carry the mecA gene or that produce PBP 2a (the mecA gene product) should be reported as oxacillin/methicillin resistant.

S. aureus that are multidrug resistant should be suspected of being MRSA and zones of inhibition around Oxacillin 1 µg Neo-Sensitabs should be examined for the presence of a light film of growth within the zone of inhibition (if found report R). Cefoxitin is easier to interpret.

Community acquired MRSA (**CA-MRSA**) infections appear to be an emerging phenomenon worldwide (11,21). They are susceptible to numerous drugs: gentamicin, chloramphenicol, trimethoprim+sulfa, fosfomycin, rifampicin etc. They are mecA positive and can be detected using Cefoxitin Neo-Sensitabs (R).
The Vitek 2 system failed to detect MRSA strains having the community acquired MRSA phenotype. Such errors may remain undetected in laboratories exclusively using broth-based methods (vitek 2, Microscan) to test the susceptibility of *S. aureus* isolates. Disk diffusion and E-test detected methicillin-resistance (26).

Warning: Methicillin (Oxacillin) resistant *S. aureus* and coagulase negative staphylococci (CNS) should be reported as resistant to all beta-lactams, (including beta-lactamase inhibitor combinations and imipenem) regardless of in vitro test results with those agents (2). Do not use cephalosporins or carbapenem disks for susceptibility testing of staphylococci. Use Cefoxitin and Oxacillin 1 µg Neo-Sensitabs.

BORSA, **Borderline oxacillin resistant *S. aureus***
Some strains of *S. aureus* with high ß-lactamase activity and mecA negative may show resistance to oxacillin. These strains are not clinically resistant. Testing with Cefoxitin Neo-Sensitabs (zone S ≥ 25 mm) will show that the strains are mecA negative (cefoxitin susceptible).

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b) Detection of strains with decreased susceptibility to Vancomycin (VISA/GISA, hVISA)

Strains of CNS showing intermediate and resistant MIC's towards vancomycin and teicoplanin have been described (5). Strains of *S. aureus* with decreased susceptibility to vancomycin (MIC 4 to 8 µg/ml) were reported from Japan (6) in 1997, and shortly thereafter from USA and France. To date all *S. aureus* strains appear to have developed from MRSA.

Automated commercial tests poorly recognize VISA, hVISA and VRSA isolates, which necessitates the use of espensive supplemental screening tests (39).

According to Liu (23) classification of strains with reduced susceptibility to vancomycin may be as follows:

1) **hVISA**: Vancomycin heteroresistant containing a susceptible population and subpopulations of VISA. Stage that precedes the development of VISA. Shows also reduced susceptibility to teicoplanin (Ex Mu 3 strains of Hiramatsu)
2) **VISA**: Shows MICs to vancomycin of 8 to 16 µg/ml and reduced susceptibility to teicoplanin (Ex Mu 50 from Hiramatsu).
3) **VRSA**: Shows MICs to vancomycin of > 32 µg/ml.

We should be aware of the following:

- VISA may not appear on the primary culture plate until day 2 of incubation (48 h).
- VISA grows more slowly than typical vancomycin-susceptible *S. aureus*.
- VISA isolates may demonstrate variable colony morphologies.
- Some colonies might be present inside the inhibition zone of the glycopeptides.
- Vancomycin and teicoplanin inhibition zones show a **sharp edge** if staphylococci are susceptible. With VISA/GISA strains the edge of the zone of inhibition around Vancomycin 5 µg and Teicoplanin Neo-Sensitabs is currently **hazy**.
- Most VISA lack the ability to hemolyze sheep blood (38).

Howden et al (30) showed that the addition of 5 % horse blood or 20 % horse serum to Brain Heart Infusion and incubation for 24-48 hours allowed highly sensitive dectection of clinically important hVISA.

The current diffusion method with vancomycin and teicoplanin disks or tablets is **not** useful for detecting VISA/GISA/ hVISA strains. Consequently a modification of the method is necessary. Using horse blood added to the medium (as recommended by Howden) and a 20 hours' prediffusion technique, Nielsen & Casals (32) obtained a clear separation between VISA/GISA/hVISA strains and VSSA. The technique is described below:

Prediffusion procedure for detecting VISA, hVISA

a) Screening of suspicious strains
MRSA strains showing zones of inhibition < 15 mm around Cefoxitin 60 µg Neo-Sensitabs (indicating high level resistance to oxacillin) in the current diffusion test should be suspected of possibly being VISA/hVISA.

b) Confirmation
One tablet each of Vancomycin 5 µg and Teicoplanin 30 µg Neo-Sensitabs are placed on an uninoculated plate containing Brain Heart Infusion Agar + 5 % horse blood.
After 2 hours at room temperature the tablets are removed (by knocking the plate against the table) and the plate is maintained at room temperature for further 18 hours (overnight).
The plate is now inoculated with the suspicious strain using a 0.5 McFarland inoculum and the plate is incubated at 33-35 °C overnight. Reincubation for further 24 hours is seldom necessary. The zones of inhibition are measured and compared to the following:

c) Interpretation
VISA/GISA/hVISA strains will show the following tentative zones of inhibition:

Teicoplanin 30 µg Neo-Sensitabs: Inhibition zone < 20 mm and/or

Vancomycin 5 µg Neo-Sensitabs: Inhibition zone < 22 mm

Practically all VISA, hVISA (GISA) strains show higher MIC's (and consequently higher resistance) to teicoplanin than to vancomycin. As a consequence, the prediffusion test with Teicoplanin 30 µg Neo-Sensitabs has the highest sensitivity and accuracy identifying these strains. Confirmation of MIC by a reference laboratory is recommended.

Recently MSSA-GISA strains have been isolated in France (36).

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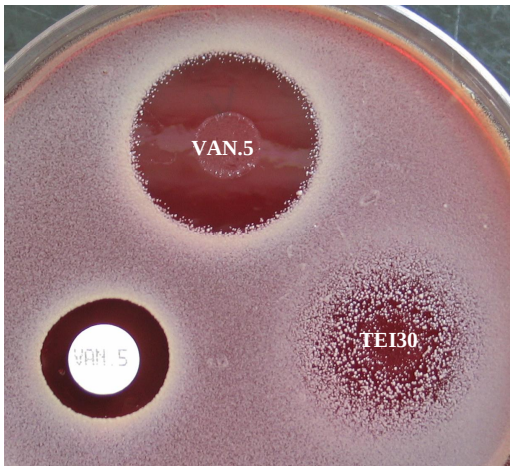

Plate 13.1-a
Staphylococcus aureus ATCC 700698 (VISA). Demonstration of prædiffusion 2+18 hours (see procedure).
Vancomycin 5 µg and Teicoplanin 30 µg Neo-Sensitabs have been removed. Note the hazy edge around Vancomycin (VAN.5) and no clear zone for Teicoplanin (TEI30). After prediffusion inoculation and one Vancomycin 5 µg has been placed for comparison of the zone. Incubated at 33-35 °C overnight.

Sandberg et al (31) in a screening study for hVISA/VISA in MRSA isolates from Denmark (1/1 2003 to 31/7 2004) reported the presence of VISA/hVISA in Denmark.
The clinical significance of hVISA strains is still controversial. It is difficult to evaluate because conventional susceptibility tests including MIC determination cannot detect hVISA (28,42).
Clinicians should bear in mind that an inadequate response to vancomycin in the treatment of MRSA infections could be due to heteroresistance to vancomycin.
An interesting property of the VISA isolates is the gradually decreasing growth rate of the bacteria, which seemed to parallel the increase in the vancomycin MIC (29).
Sakoulas et al. (38) showed that GISA/VISA strains have a decreased hemolysin expression and lack the ability to hemolyze blood on sheep blood agar plates.

The MIC of daptomycin is higher for GISA than for susceptible strains, despite a different mechanism of action or resistance between vancomycin and daptomycin. It is attributed to the fact that daptomycin being a large molecule has difficulty in passing through the thickened cell wall (41).

Vancomycin resistant *S. aureus* (VRSA)

The first documented case of infection caused by vancomycin resistant *S. aureus* (VRSA) with vancomycin MIC > 32 µg/ml in a patient in USA has been described (15,17,19). The strain could be detected using the disk diffusion procedure (van A gene).

Tenover et al (24) describe the second VRSA strain in the USA (vancomycin MIC 32 µg/ml). The strain was detected using disk diffusion and vancomycin agar screen, while automatic systems (Vitek2 and Microscan) were unable to detect vancomycin resistance.

The third VRSA (25) with a van A gene was detected in New York (vancomycin MIC > 64 µg/ml). Commonly used automatic methods (Microscan, Vitek) failed to detect vancomycin resistance in this VRSA strain, while agar-based methods detected vancomycin resistance.

The 4th US case of VRSA (Michigan) has been confirmed by the CDC (March 2005). The strains showed an MIC ≥ 256 µg/ml by broth microdilution and E-test. Vitek 2 failed to detect resistance with a reported MIC of ≤ 1 µg/ml (28).

Consequently, additional VRSA infections might have occurred but were undetected by laboratories using only automated methods.

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13.2 Coagulase Negative Staphylococci

Concerning methicillin resistance in CNS, the proportion of resistant bacteria in the heterogenous population is lower than for *S. aureus*, making the detection problems more difficult to solve (special interpretation required) (10).
For susceptibility testing of CNS except *S. lugdunensis*, we recommend:

- Direct inoculum (suspension of colonies from an overnight culture in 0.9 % saline, McFarland 0.5).
- Diffusion test with **Cefoxitin** Neo-Sensitabs and Oxacillin 1 µg - Neo-Sensitabs incubated at 33-35 °C on Mueller-Hinton agar (without NaCl added).
- Incubation for 24 hours, with reincubation for a total of 48 hours, in doubtful cases.
- Interpretation: For Cefoxitin 60 µg use S ≥ 28 mm (oxacillin S) and R ≤ 27 mm (mecA positive, oxacillin R).
  S: ≥ 18 mm (MIC ≤ 0.25 µg/ml) and R ≤ 17 mm (MIC ≥ 0.5 µg/ml) for Oxacillin 1 µg.
  MecA negative isolates that show MICs ≥ 4 µg/ml towards oxacillin should be reported as oxacillin resistant (NCCLS 2003).

Testing of oxacillin against urine isolates of ***S. saprophyticus*** is not recommended, because mecA-negative strains of *S. saprophyticus* often appear resistant by interpretative criteria used for coagulase-negative staphylococci (M100-S11, NCCLS 2001). In general, routine testing of urine isolates of *S. saprophyticus* is not advised, because infections respond to antimicrobial agents commonly used to treat acute, uncomplicated urinary tract infections (2).
Cefoxitin Neo-Sensitabs can be used to detect mec A positive strains of *S. saprophyticus*. For *S. lugdunensis* see recommendations for *S. aureus* (Chapter 13.1).

Methicillin resistant CNS are even more resistant to multiple antibiotics than *S. aureus*. The observation of multiple resistance should be a clue to the possibility of methicillin resistance. NaCl should not be added in the diffusion test, while MIC tests (broth and agar dilution) give optimal results with Mueller-Hinton added of 2% NaCl and incubation at 33-35 °C for a full 24 hours.

Mackenzie et al. found discrepancies in the Oxacillin 1 µg disk test results given by Mueller-Hinton agars from three manufacturers, when testing against *S. aureus* with low-expression-class methicillin resistance. They recommend to use a low-expression-class methicillin resistant *S. aureus* as a control for the medium used (3). Coombs et al. (4) had problems detecting low-expression-class methicillin resistance in *S. aureus* with several batches of Oxoid Mueller-Hinton agar.

Vancomycin-resistant staphylococci (*S. haemolyticus*, *S. epidermidis* and *S. capitis*) have been isolated from healthy carriers in Brazil (33). For detection use the 20 hours' prediffusion technique recommended for VISA/hVISA.

13.3 Comments Concerning Other Antimicrobials

Penicillin susceptible staphylococci are also susceptible to other penicillins, cephems and carbapenems. Thus susceptibility or resistance to a wide range of beta-lactams may be deduced from testing only Penicilin Low, Cefoxitin and Oxacillin 1 µg Neo-Sensitabs.

Some rare strains of *S. aureus* produce low levels of ß-lactamase. They may be identified as penicillin resistant because the edge of the zone around Penicillin Low Neo-Sensitabs is **sharp.**

Staphylococcus spp. may develop resistance during prolonged therapy with quinolones. Susceptible isolates may become resistant within 3-4 days after initiation of treatment. Testing of repeat isolates may be warranted (11).

Rifampicin and fusidate should not be used alone for therapy (development of resistance) (44).

MRSA treatment failures occurred in children when clindamycin was used to treat inducible MLS_B resistance (Chavez-Bueno (20), Siberry et al. (22)).

For the detection of Erythromycin resistance phenotypes use the double tablet induction test, also described for streptococci in page 117 (inducible MLS_B, constructive MLS_B, efflux).

Kanamycin/Amikacin: Strains possessing the enzyme AAC (3^1)-III may show only a minor increase in the MIC of amikacin, but the bactericidal and synergistic activities of amikacin (with beta-lactams or glycopeptides) disappear. Consequently routine testing of kanamycin (not amikacin) against all staphylococci should be performed and the results reported as amikacin (41).

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Double tablet induction test (D Zone-test). Inducible clindamycin resistance
Clindamycin and Erythromycin Neo-S are placed approx. 20 mm apart (from edge to edge). Following incubation organisms showing **flattening** of the clindamycin zone near the Erythromycin tablet indicate inducible clindamycin resistance. Such isolates should be reported as **clindamycin resistant** (NCCLS 2004).
Inducible clindamycin resistance is not detected by current susceptibility test methods including automatic methods. When using Erythromycin 15 µg and Clindamycin 2 µg, the distance between disks must be ≤ 15 mm (40).

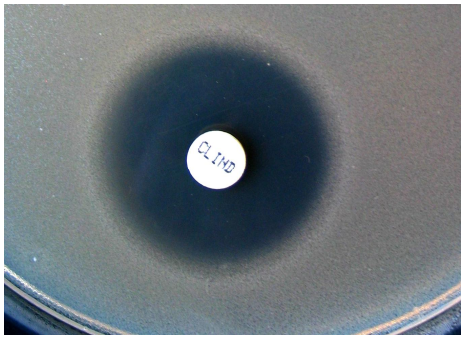

Plate 13.3-a

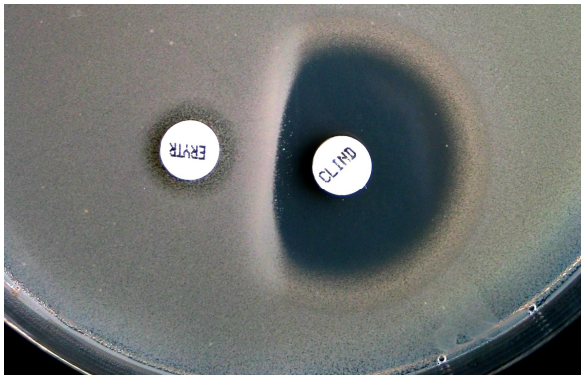

Plate 13.3-b

Demonstration of the presence of inducible clindamycin resistens in *Staphylococcus aureus* (inducible MLS_B resistance). Note the flattening of the Clindamycin zones (Plate 13.3-b) near the Erythromycin Neo-Sensitabs (ERYTR) and the big circular zone (Plate 13.3-a) around the Clindamycin Neo-Sensitabs (CLIND) when not induced.

CA-MRSA are often susceptible to non-beta-lactam drugs, althoug they may show resistance to macrolides by an efflux mechanism. These strains should be tested for inducible clindamycin resistance (27).

Differentiation of phenotypes of macrolide resistance in staphylococci may be possible by the following criteria:

Phenotypes of macrolide resistance in staphylococci (34,35)

Phenotype	Genotype	Induction test type	CLINDA	ERY	Remarks
Inducible MLS_B	erm A	D	S (→R)	R	Blunted D-shaped clear zone around CLINDA tablet, near the ERY tablet. Report CLINDA resistant.
Inducible MLS_B	erm C	D^+	S (→R)	R	Blunted D-shaped zone around CLINDA tablet near the ERY tablet and small colonies growing into the CLINDA zone in an otherwise clear zone. Report CLINDA resistant.
Constitutive MLS_B	erm A	Hazy D	R	R	Some times 2 zones of growth appear around the CLINDA tablet. An inner zone of light growth and an outer zone of confluent growth. The inner zone is blunted, near to the Ery tablet. Report CLINDA resistant.
Constitutive MLS_B	erm A erm A+C	R	R	R	No hazy zone. Growth up to both the ERY and CLINDA tablets.
MS_B (M)	mef (A) msr A	negative	S	R	Clear susceptible zone around the CLINDA tablet. Report CLINDA susceptible.
Constitutive **L**	lin A	negative	I/R	S	Clear susceptible zone around ERY. No zone around CLINDA or reduced zone size (< 20 mm). Report ERY susceptible and CLINDA resistant.
No resistance	none	S	S	S	Clear susceptible zone diameter.

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13.4 MRSA Quality Control

***S. aureus* ATCC 43300**

	NEO-SENSITABS	POTENCY	CODE	Zone diameter in mm	
	Amoxycillin+Clavulanate	30+15 µg	AM+CL	22-25	(R)
b)	Cefoxitin	60 µg	CFOXT	16-20	(R)
	Ciprofloxacin	10 µg	CIP10	23-24	(S)
	Erythromycin	78 µg	ERYTR	9	(no zone)
	Gentamicin	40 µg	GEN40	16-22	(I/R)
	Linezolid	30 µg	LINEZ	26-32	(S)
a)	Oxacillin	1 µg	OXA.1	9	(no zone)
	Tetracyclines	80 µg	TET80	32-39	(S)
	Trimethoprim	5.2 µg	TRIME	24-31	(S)
	Vancomycin	**5 µg**	VAN.5	18-23	(S)
	Vancomycin	**70 µg**	VAN70	22-27	(S)

a) There is a zone around Oxacillin 1 µg, but it is overgrown of thin colonies. The zone should be read as 9 (resistant).

b) The strain is also resistant to Cefoxitin.

13.5 VISA / GISA Quality Control

Using the 20 hours' (2+18 h) prediffusion technique, recommended for detection of VISA/GISA/hVISA strains on Brain Heart Infusion **5 % horse blood** and inoculum 0.5 McFarland, Q.C. results should be as follows:

Strains: *S. aureus* ATCC 700788 (VISA)
S. aureus ATCC 43300 (VSSA)

	Teicoplanin 30 µg 2+18 h prediffusion		Vancomycin 5 µg 2+18 h prediffusion	
	Zone Diameter	**MIC µg/ml**	**Zone Diameter**	**MIC µg/ml**
S. aureus ATCC 70078	9-12	16	15-20	4-8
S. aureus ATCC 43300	23-27	0.5	23-27	1

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14 Detection of Resistant Enterococci

Enterococci should always be tested on plain agar **without** blood. Use of blood containing media in susceptibility testing of enterococci may result in **false** susceptibility results with aminoglycosides, cefotaxime and other cephalosporins (1,2,3).

14.1 Penicillin/Ampicillin Resistance

Enterococci may be resistant to penicillin/ampicillin because of production of low affinity PBPs or less commonly due to the production of beta-lactamase (**sharp** edge of the zone of inhibition). The Ampicillin 33 µg Neo-Sensitabs diffusion test accurately detects isolates with altered PBPs. The rare betalactamase-producing strains are best detected using a direct nitrocefin test (4). The majority of *E. faecium* are resistant to ampicillin (low affinity PBPs).

Enterococci (*E. faecium*) with high level resistance to ampicillin (MIC ≥ 64 µg/ml ~ zone ≤ 12 mm) may not be susceptible to the synergistic effect with aminoglycosides.

Ampicillin susceptibility is used to predict the susceptibility of amoxycillin, acylampicillins, ampicillin+sulbactam, amoxycillin+clavulanate, piperacillin and piperacillin+tazobactam for non-beta-lactamase producing enterococci (cross-resistance).

An unusual resistance phenotype (16) was recently detected in 33 nosocomial strains of *E. faecalis* in a Greek hospital. The strains were resistant to penicillin (MIC > 32 µg/ml) and susceptible to ampicillin and amoxycillin+clavulanate (MIC 0.4-1.5 µg/ml). The same resistance phenotype has been observed in Denmark (20).

Weinstein (9) recommends the use of ampicillin (surrogate disk) as representative for imipenem, when testing against *E. faecalis* and *E. faecium*. Recently, some ampicillin-sensitive, imipenem-resistant strains have been isolated in Europe (10). Consequently sensitivity testing with imipenem should be done when enterococcal infections with this drug is considered for treatment.

Ampicillin susceptible (MIC ≤ 2 µg/ml) but penicillin (MIC ≥ 16 µg/ml) and imipenem (MIC ≥ 8 µg/ml) resistant strains of *E. faecalis* were widely disseminated (31.4 % of the *E. faecalis* with this phenotype) in a Greek hospital (22).

Ono et al (19) reported ampicillin (MIC 8-16 µg/ml) and imipenem (MIC 4-32 µg/ml) resistant isolates of Van A *E. faecalis*. Resistance is due to point mutations of PBP4.

It should be emphasized that bactericidal therapy of serious enterococcal infections such as endocarditis, can only be achieved with combined drug therapy (high dose penicillin/ampicillin or vancomycin + aminoglycoside) but combined therapy is not required for less serious infections, e.g. UTI (4).

14.2 Glycopeptide Resistance (VRE)

Accurate detection of vancomycin-resistant enterococci by the diffusion method, requires the following:

- Use of **Vancomycin 5 µg** Neo-Sensitabs on Mueller-Hinton agar without blood and McFarland 0.5 inoculum.
- Use the 2+18 hours' prediffusion method.
- Examine carefully the vancomycin zone of apparent inhibition with transmitted light for evidence of **small colonies** or **light film** growing within the zone. If found, report resistance to vancomycin.
- Use the interpretation S: > 16 mm, R: < 16 mm.
- It is important to examine the **zone edges** when reading the zone diameters around Vancomycin Neo-Sensitabs. Vancomycin susceptible strains **show sharp** edges, while vancomycin resistant strains show **diffuse edges**.
- See Quality Control Limits with *E. faecalis* ATCC 51299 (see chapter 14.5).

Warning:
Vancomycin testing on Iso-sensitest Agar and Danish Blood Agar will not detect **vanB** resistance and therefore should be avoided.

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Resistance to glycopeptides in enterococci has been detected in different species in many countries. Phenotypically, this resistance has been divided into several phenotypes. See table page 94.

The results are interpreted as follows:

Compare the test strain with a Reference sensitive strain (*E. faecalis* ATCC 29212):

1) An inhibition zone **smaller** than the Reference strain with a **sharp edge** is typically *E. gallinarum / E. casseliflavus*.
2) The test strain show a zone of inhibition similar to that of the Reference strain and a **sharp edge**. It is sensitive to vancomycin.
3) The test strain has a zone of inhibition with a **hazy edge** i.e. fine growth visible at the edge of the zone. The isolate is VRE with van B type of resistance.

Van A: Strains characterized by an inducible high level resistance to both vancomycin (VANCO) and teicoplanin (TEICO). Both vancomycin and teicoplanin are resistance inducers, although vancomycin has a higher inducer activity.

These strains show no zone of inhibition around Vancomycin 5 µg Neo-Sensitabs (current diffusion method).

The Van A resistance is generally plasmid-mediated and may be transferred by conjugation to susceptible strains. It should be expected that the Van A resistance will spread from enterococci to other microorganisms (5).

Van B: Strains characterized by variable levels of resistance to vancomycin with MICs 16-1024 µg/ml. The strains are susceptible to teicoplanin with MICs 0.5-1 µg/ml. Resistance is only induced by vancomycin, but once the strains have been induced, they show resistance to both VANCO and TEICO. The Van B phenotype can also be transferred by conjugation.

These strains show with the 2+18 hours' prediffusion method: zone < 16 mm and hazy edge.

Van C: Strains characterized by a constitutive low level resistance to vancomycin, but being susceptible to teicoplanin.

Strains of phenotype Van C are less frequently encountered species of **motile** enterococci. They can be detected by performing a **motility** test. Motile enterococci show either intrinsic low level resistance to vancomycin (zone around Vancomycin 5 µg Neo-Sensitabs) or have acquired high level resistance (no zone around Vancomycin 5 µg Neo-Sensitabs, Van A phenotype).

These strains show with the 2+18 hours' prediffusion method: zone < 16 mm and sharp edge.

E. casseliflavus, *E. flavescens* and *E. gallinarum* with vancomycin MIC's of 8-16 µg/ml (intermediate) differ from vancomycin resistant enterococci for infection control purposes (NCCLS 2000).

Heterogeneous phenotype:
Recently, new resistant phenotypes: Van D, Van E have been described (7). Rabiul Alam M. et al (8) describes **heteroresistance** to Vancomycin in *E. faecium*. The diffusion test with Vancomycin 5 µg Neo-S, will show growth af subcolonies inside the zone of inhibition.

Van B phenotype – Van A genotype *E. faecium* with heterogeneous expression of glycopeptide resistance have been isolated from a Korean hospital (Vanco MIC 64-128 µg/ml and Teico MIC 4-12 µg/ml) (26,27).

Glycopeptide-dependent strains:
Glycopeptide-dependent strains require vancomycin/teicoplanin for growth (21). They are currently resistant to vancomycin or to both vancomycin and teicoplanin and appear as colonies growing up to the edge of the vancomycin tablet (disk).

When testing Van D isolates comparing semiconfluent growth and McF. 0.5 inoculum respectively, a large difference in zone size will be obtained (10 mm or more) due to the high inocolum effect (11).

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Characteristics (5,7,21) **(A) Acquired resistance**

	High Level		Variable level		
Phenotype	Van A	Van B	Van D (11)	Van E (13)	Van G (15)
MIC µg/ml					
Vancomycin	≥ 64	(4)-16-1024	(16)-64-128	6-16-32	16
Teicoplanin	≥ 16	0.5-1	(0.25)-4-64	0.5	0.5
Transfer by conjugation	+	+	0	0	+
Resistance induced by					
Vancomycin	+	+	0	+	+
Teicoplanin	+	0	0	0	0
Location	Plasmid-chromosome	Plasmid-chromosome	Chromosome	Chromosome	Chromosome
Microorganisms detected	*E. faecium* *E. faecalis* *E. durans* *E. gallinarum* (14) (MIC Vanco 32-128 µg/ml) *E. casseliflavus* *E. mundtii* *E. avium* *E. hirae* *E. raffinosus*	*E. faecium* *E. faecalis* *E. gallinarum*	*E. faecium* *E. faecalis* high inoculum effect *E. gallinarum* *E. raffinosus*	*E. faecalis*	*E. faecalis*

Characteristics (5,7) **(B) Intrinsic resistance**

	Low level		High level
Phenotype	Van C		
MIC µg/ml	C_1	C_2 / C_3	
Vancomycin	8-16	4-32	≥ 1000
Teicoplanin	0.5	0.5-1	≥ 250
Transfer by conjugation	0	0	0
Resistance induced by			
Vancomycin	0 or +	ND	0
Teicoplanin	0	ND	0
Location	Chromosome	Chromosome	
Microorganisms detected	*E. gallinarum*	*E. casseliflavus* *E. flavescens*	Lactobacillus *Leuconostoc* spp. *Pediococcus* spp. *E. rhusiopathiae* *Lactococcus* spp.

If vancomycin is used for serious enterococcal infections, such as endocarditis, combined therapy with an amino-glycoside is usually indicated (4).

Hospital outbreaks of VRE (van A, van B) are almost exclusively caused by a specific genogroup of VR *E. faecium* that can easily be characterized by co-resistance to ampicillin (Vanco I/R, Ampi R) and the presence of the variant **esp** gene (18). Ampicillin resistance (MIC ≥ 16 µg/ml and/or zone < 20 mm with Ampicillin 33 µg Neo-S) appears to be a specific and sensitive marker for this genogroup.

In vivo transfer of a Van A resistance gene from an animal isolate of *E. facecium* into a human isolate of *E. faecium* has been achieved in the intestines of human volunteers (23).

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14.3 High-level Aminoglycoside Resistance (HLR)

High level resistance to aminoglycosides is an indication that an enterococcal isolate will not be affected synergistically by a combination of a penicillin or glycopeptide plus an aminoglycoside (4).

Screening for high level gentamicin and streptomycin resistance should be performed on enterococcal isolates from blood or CSF. Special, high-content Neo-Sensitabs: Gentamicin 250 µg, Kanamycin 500 µg and Streptomycin 500 µg are used to screen for this type of resistance.

Inhibition zones < 14 mm indicates high level resistance (HLR) and zones ≥ 15 mm indicates a lack of HLR. The test is performed on plain Mueller-Hinton agar using McFarland 0.5 inoculum:
If the inoculum used results in semiconfluent growth, the zone diameter breakpoint is 17 mm (instead of 14 mm).

Aminoglycosides	Zone diameters in mm High level resistant (HLR)	Equivalent Break-point MIC µg/ml
Gentamicin 250 µg	< 14 mm (HLR)	> 500
Kanamycin 500 µg	< 14 mm (HLR)	> 1000
Streptomycin 500 µg	< 14 mm (HLR)	> 1000

- If the strain is HLR to streptomycin: This aminoglycoside cannot be used in combination with a penicillin or glycopeptide.
- If the strain is HLR to kanamycin: Then kanamycin, isepamicin and amikacin cannot be used.
- If the strain is HLR to gentamicin: Then the strain is HLR to all **aminoglycosides** (gentamicin, tobramycin, sisomicin, netilmicin, kanamycin, isepamicin and amikacin), except streptomycin.

HLR to gentamicin in enterococci is known to be mediated by the bifunctional enzyme AAC(6')-APH(2'').

E. faecium is known to harbour a chromosomally mediated enzyme AAC(6')-1 and consequently, is naturally resistant to penicillin-tobramycin, penicillin-netilmicin, penicillin-sisomicin and penicillin-kanamycin synergy (6). For *E. faecium* report HLR to all aminoglycosides except gentamicin and streptomycin.

Recently (17) new *aac (6')-Ii*-like genes have been characterized in *E. hirae* and *E. durans*, which precludes the synergy between tobramycin or kanamycin/amikacin and beta-lactams, even if the strains are not HLR. These results indicate that except for *E. faecalis*, association of ß-lactams with tobramycin and kanamycin/amikacin should not be recommended in the therapy of enterococcal infections.

Penicillin-gentamicin synergy may be obtained even in *E .faecium* strains exhibiting penicillin/ampicillin resistance, provided that appropriate penicillin concentrations are used (in most cases half of the penicillin MICs) (6).

14.4 Comments concerning Other Antibacterials

High level resistance to ciprofloxacin (MIC ≥ 64 µg/ml) appears to be associated with ampicillin resistance in genotypically related *E. faecium* isolates, and favours the hospital adaptation of lineage CC17 of *E. faecium* (24).

New antimicrobials: linezolid, quinupristin/dalfopristin (*E. faecium* only) show good activity against enterococci.

A relatively high rate (40 %) of VR *Enterococcus faecium* not susceptible (MIC ≥ 8 µg/ml) to linezolid was observed recently in Italy, by Bonosa et al. (25) in intensive care unit patients.

Because of limited alternatives, chloramphenicol, erythromycin, doxycycline/minocycline and rifampicin may be tested for vancomycin-resistant Enterococci (VRE) and consultation with an infectious disease practitioner is recommended (NCCLS 2001).

Cephalosporins, aminoglycosides, clindamycin and trimethoprim+sulfa should not be tested and/or reported against enterococci. Reporting of these results can be dangerously misleading , except for screening for high-level aminoglycoside resistance (4).

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Double tablet induction test (D zone-test) (12)
For the detection of macrolide resistance phenotypes in enterococci, use the double tablet induction test described for streptococci in page 117 . Instead of Clindamycin (enterococci are resistant) use Tylosin (16 membered ring) and Erythromycin Neo-Sensitabs at a distance of 10 mm between the tablets.
Absence of significant zone of inhibition around the two Neo-Sensitabs indicates: constitutive MLS_B resistance ($cMLS_B$). Blunting of the Tylosin **or** of the Erythromycin zones indicates inducible MLS_B resistance ($iMLS_B$).
The $iMLS_B$ phenotype with blunting of erythromycin zone near the Tylosin tablet indicates reversibly inducible MLS_B resistance ($riMLS_B$).

14.5 Enterococci HLR and VRE Quality Control

***E. faecalis* ATCC 51299 (van B) ***

			Zone diameter in mm		
NEO-SENSITABS	**POTENCY**	**CODE**	***E. faecalis* ATCC 51299**		**MIC µg/ml**
AMOXYCILLIN	30 µg	AMOXY	25-30		
AMPICILLIN	33 µg	AMP33	25-31		
CHLORAMPHENICOL	60 µg	CLR60	9-14	(R)	
DOXYCYCLINE	80 µg	DOXYC	26-32		
ERYTHROMYCIN	78 µg	ERYTR	no zone	(R)	
GENTAMICIN	250 µg		no zone	(HLR)	> 2000
LINEZOLID	30 µg	LINEZ	23-29	(S)	
PENICILLIN LOW	5 µg	PEN.L	12-15		
RIFAMPICIN	30 µg	RIFAM	21-26		
STREPTOMYCIN	500 µg		no zone	(HLR)	> 2000
TEICOPLANIN	60 µg	TEICO	17-23	(S)	
TETRACYCLINES	80 µg	TET80	24-30		
VANCOMYCIN	5 µg	VAN.5	12-14	(I/R)	16-32

* MH-agar, inoculum McF 0.5, incubation 35 °C 16-18 hours (24 hours for vancomycin).

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15 Susceptibility Testing of Fastidious or Problem Organisms

The CLSI (Kirby-Bauer) method and other diffusion tests have been standardized for rapidly growing pathogens. Larger zones of inhibition will result, if the test is performed with organisms that have a slow rate of growth, resulting in erroneous results in the susceptibility test. Consequently, it is important to give optimal growth conditions to the strains being tested. This may be achieved by using:

a) A lower incubation temperature
It is well-known that methicillin-resistant strains of staphylococci sometimes are reported as susceptible to methicillin by disk testing at 37°C, but as resistant at 30-35°C (or with NaCl added to the medium). This phenomenon is attributed to the heterogeneity of the bacterial population, the resistant part of the population having an optimal growth temperature at 30-35°C, is not detected at 37°C, because of poor (slower) growth.

Strains showing a better growth rate at 30°C than at 37°C, should be tested at 30°C (optimal growth temperature). The antibiotic diffusion from Neo-Sensitabs is not affected significantly by using incubation temperatures below or above 37°C. The following species (1) may show a better growth at 30°C: *Yersinia* spp., *Klebsiella ozaenae*, certain non-fermentative gram-negative rods: *Stenotrophomonas maltophilia*, *Pseudomonas putida*, *Pseudomonas fluorescens*, some strains of *Acinetobacter* spp., *Burkholderia cepacia*, *Aeromonas* spp., and some *Moraxella* spp.

b) Nutritionally supplemented media (2)
Some strains require supplemented media for their growth:

1) Symbiotic streptococci, responsible for bacterial endocarditis require pyridoxine, thiol or Isovitalex.

2) Strains of Enterobacteriaceae which form dwarf colonies on media (e.g. *E. coli*, *Citrobacter* spp., *Klebsiella* spp., *Proteus* spp., *Salmonella* spp.) require supplement nutrients for larger colony growth. Among these are the thymineless variants, most likely related to the therapeutic use of trimethoprim. Supplementation of the medium with thymidine is required for correct testing of these organisms. Other strains require CO_2, thiamin, glutamic acid etc.

3) Strains of *S.aureus* that form dwarf colonies in routine media require supplement with thiamin and/or menadione, for normal growth.

Some of the supplemental substances may interfere with the activity of the antibiotics, e.g. CO_2 affects the activity of aminoglycosides, macrolides, and tetracyclines, in which case a modification of the zone size interpretation may be necessary. Strains that require thymidine must be tested on media supplemented with thymidine, because the effect of thymidine on trimethoprim can be disregarded since such organisms are resistant to trimethoprim in vitro and in vivo.

c) Species specific breakpoints
When testing the susceptibility of slow growing organisms and/or species with special requirements (*Haemophilus* spp., *Neisseria* spp., pneumococci, streptococci) the methods must be modified to fit each organism and special interpretation tables are required (3). Special methodologies may be required to detect problem organisms such as methicillin-resistant staphylococci, resistant enterococci and extended spectrum beta-lactamase producing gram negative bacilli. Methodologies (including interpretation tables) are described in the following pages.

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15.1 Susceptibility Testing of *Haemophilus influenzae* and *H. parainfluenzae*.

The medium recommended for diffusion testing of *Haemophilus* spp. is Haemophilus Test Medium (HTM). It consist of the following ingredients: Mueller Hinton agar, 15 µg/ml NAD, 15 µg/ml bovine hematin, 5 mg/ml yeast extract + pH adjusted to 7.2 - 7.4.
To make HTM: 50 mg bovine hematin is dissolved in 100 ml 0.01 N NaOH with heat and stirring. 30 ml of this solution are added to 1 l MHA with 5 g yeast extract. Autoclave, cool to 45-50 °C and add aseptically 3 ml of NAD sol. (50 mg NAD dissolved in 10 ml H_2O and filter sterilized). pH adjusted to 7.2 - 7.4.

Procedure (1)
Colonies are taken directly from an overnight (20-24 h) chocolate agar culture and a suspension prepared in broth or 0.9% saline. The suspension is adjusted to a 0.5 McFarland standard using a photometer. Within 15 min. after adjusting the turbidity of the suspension, it should be used for plate inoculation. Notice that too high inoculum may lead to false-resistant results with some beta-lactam antibiotics.

Apply in general no more than 9 Neo-Sensitabs to the surface of a 140-150 mm plate and no more than 4 Neo-Sensitabs on a 90-100 mm plate.

Incubate at 35°C in an atmosphere of 5-7% CO_2, for 16-18 h., before measuring the zones of inhibition. The zone margin should be considered as the area showing no obvious growth visible with the unaided eye. Faint growth, or tiny colonies that may appear to fade from the more obvious zone, should be ignored in the measurement (1).

Zone diameter interpretative criteria when testing *Haemophilus* spp. are listed in the table below.

***Haemophilus* spp.**
HTM-agar, Inoculum: McFarland 0.5, Incubation in 5-7 % CO_2,
Break-points according to CLSI (M2-A8)

	NEO-SENSITABS	POTENCY	CODE	Zone diameter in mm S	I	R	Break-points MIC µg/ml S	R
a)	Amoxycillin+Clav.	30+15 µg	AM+CL	≥ 24	-	< 24	≤ 4+2	≥ 8+4
a)	Ampicillin	**2.5 µg**	AMP.L	≥ 20	19-17	≤ 16	≤ 1	≥ 4
a)	Ampicillin	**33 µg**	AMP33	≥ 28	27-24	≤ 23	≤ 1	≥ 4
a)	Ampicillin+Sulbactam	30+30 µg	AM+SU	≥ 26	-	< 26	≤ 2+1	≥ 4+2
b)	Azithromycin	30 µg	AZITR	≥ 16	-	-	≤ 4	-
b)	Aztreonam	30 µg	AZTRM	≥ 28	-	-	≤ 2	-
a)	Cefaclor	30 µg	CCLOR	≥ 23	22-20	≤ 19	≤ 8	≥ 32
	Cefepime	30 µg	CFEPM	≥ 28	-	-	≤ 2	-
b)	Cefixime	30 µg	CFFIX	≥ 32	-	-	≤ 1	-
b)	Cefotaxime	30 µg	CFTAX	≥ 28	-	-	≤ 2	-
	Cefpirome	30 µg	CFPIR	≥ 28	-	-	≤ 2	-
b)	Cefpodoxime	30 µg	CFPOX	≥ 28	-	-	≤ 2	-
b)	Ceftazidime	30 µg	CEZDI	≥ 28	-	-	≤ 2	-
b)	Ceftriaxone	30 µg	CETRX	≥ 28	-	-	≤ 2	-
a)	Cefuroxime	60 µg	CEFUR	≥ 28	27-24	≤ 23	≤ 4	≥ 16
	Cephalothin	66 µg	CLOTN	≥ 28	-	≤ 23	≤ 8	(amp R)
	Chloramphenicol	**10 µg**	CLR10	≥ 20	19-17	≤ 16	≤ 2	≥ 8
	Chloramphenicol	**60 µg**	CLR60	≥ 32	31-27	≤ 26	≤ 2	≥ 8
*	Ciprofloxacin	**10 µg**	CIP10	≥ 26	-	-	≤ 1	-
	Clarithromycin	30 µg	CLARI	≥ 13	12-11	≤ 10	≤ 8	≥ 32
**	Doxycycline	80 µg	DOXYC	≥ 28	27-24	≤ 23	≤ 2	≥ 8
	Gatifloxacin	5 µg	GATIF	≥ 18	-	-	≤ 1	-
b)	Imipenem	15 µg	IMIPM	≥ 22	-	-	≤ 4	-
b) d)	Levofloxacin	5 µg	LEVOF	≥ 20	-	-	≤ 2	-
b) c)	Meropenem	10 µg	MEROP	≥ 26	-	-	≤ 0.5	-
b) d)	Moxifloxacin	5 µg	MOXIF	≥ 18	-	-	≤ 1	-

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NEO-SENSITABS		POTENCY	CODE	Zone diameter in mm S	I	R	Break-points MIC µg/ml S	R
d)	Nalidixan	130 µg	NALID	-	-	< 25	-	reduced susceptibility to quinolones
b) d)	Ofloxacin	10 µg	OFLOX	≥ 22	-	-	≤ 2	-
	Penicillin Low	5 µg	PEN.L	≥ 20	19-17	≤ 16	≤ 1	≥ 4
	Rifampicin	30 µg	RIFAM	≥ 28	27-24	≤ 23	≤ 1	≥ 4
	Telithromycin (18)	15 µg	TEL15	≥ 15	14-12	≤ 11	≤ 4	≥ 16
	Tetracyclines	**10 µg**	TET10	≥ 20	19-17	≤ 16	≤ 2	≥ 8
	Tetracyclines	**80 µg**	TET80	≥ 30	29-27	≤ 26	≤ 2	≥ 8
	Trimethoprim+Sulfa	5.2+240 µg	TR+SU	≥ 28	27-24	≤ 23	≤ 0.5/9.5	≥ 4/76
	Trimethoprim	5.2 µg	TRIME	≥ 23	22-20	≤ 19	≤ 1	≥ 4

* Special breakpoints - see 4) page 101.
** MIC breakpoints have not been established by the CLSI.

a) Beta-lactamase-negative ampicillin-resistant (BLNAR) strains are best detected using **Ampicillin 2.5 µg**. These strains should be considered resistant also to amoxycillin, amoxycillin+clavulanate, ampicillin+sulbactam, cefaclor, cefonicid, and cefuroxime despite apparent in vitro susceptibility of some BLNAR strains to these agents. Cefaclor and Cephalothin may also be used to detect BLNAR strains. Beta-lactamase negative strains resistant to cefaclor (< 19 mm) and/or cephalothin (< 23 mm) are BLNAR.

b) The CLSI has not yet defined other categories than "S" due to the current absence of resistant strains.

c) Clinical indications and relevant pathogens include bacterial meningitis and concurrent bacteremia in association with meningitis caused by H. influenzae (2).

d) Fluoroquinolone resistant *H. influenzae* are rare. Non-susceptible strains should be sent to a Reference Laboratory (2). Strains resistant to nalidixan should be suspected of having reduced susceptibility to quinolones. Strains with decreased susceptibility to ciprofloxacin have decreased susceptibility to all quinolones (7). The current CLSI, MIC breakpoints for quinolones will not always detect resistance (16).

Note: Only results of testing with ampicillin, one of the third gen. cephalosporins, chloramphenicol, and meropenem should be reported routinely with all blood and CSF isolates of H. influenzae recovered from patients with life-threating infections (e.g. meningitis, bacteremia, epiglottitis and facial cellulitis) (2).

A) Beta-lactams
When testing the susceptibility of *Haemophilus* spp. it should be possible to detect 2 different types of resistance towards ampicillin:

1) Beta-lactamase producing strains (plasmidic TEM or ROB-1).
Beta-lactamase producing strains are easily detected a) with a rapid beta-lactamase test e.g. Beta-lactamase (acido) Diagnostic Tablets (ROSCO code 455-21), using several colonies, because producers and non-producers may coexist in the sample, b) using Amoxycillin+Clavulanate Neo-Sensitabs compared to Amoxycillin alone. Synergism (larger zone with Amoxycillin+Clavulanate) will be seen in the presence of a beta-lactamase.

2) Chromosomal resistance to ampicillin (due to alteration of PBP's and/or reduction in permeability).
Zerva et al (3) showed in a study of 300 H.influenzae strains, that testing with a 2 µg ampicillin disk gave a superior interpretative accuracy than the 10 µg ampicillin disk. The latter miscategorised as susceptible or intermediate 81.3% of the BLNAR strains tested. Kärpänoja P. et al (15) found similar results. Testing of **Ampicillin 2.5 µg** - Neo-Sensitabs serves better to detect the rare BLNAR strains, than testing of Ampicillin 33 µg or of Amoxycillin+Clavulanate by the diffusion method.
Beta-lactamase negative Haemophilus, resistant to ampicillin (amoxycillin) should be reported as **resistant to combinations** with beta-lactamase inhibitors (Amoxycillin+Clavulanate, Ampicillin+Sulbactam) irrespective of zone size, because beta-lactamase inhibitors have no effect on beta-lactamase negative strains.
The prevalence of BLNAR strains is increasing in Europe (8).

17 beta-lactamase positive isolates that were resistant to amoxycillin+clavulanate were detected in a US National Surveillance Study (5). If these strains become common, it would be inappropriate to consider all beta-lactamase positive strains to be uniformly susceptible to AM+CL.

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Matic et al. (13) reported that AM+CL resistance in *Haemophilus* is due to changes in PBP3, in combination with ß-lactamase production.
Cerquetti et al. (19) describe the first heterogeneous resistance to imipenem in *H. influenzae* using E-test. The standard broth dilution method was unable to detect resistance.

Differentiation of strains showing beta-lactam resistance:

H. influenzae	**AMP 2.5**	**AM+CL**	**Beta-lactamase**	**Cefpodoxime/Clav.**
Sensitive	Zone > 20 mm	Zone S	neg.	no syn.
BLNAR	Zone < 20 mm	-	neg.	no syn.
Beta-lactamase pos.	No zone	Zone > 28 mm (S)	pos.	no syn.
AM+CL resistant	No zone	Zone < 28 mm	pos.	no syn.
ESBL positive	No Zone	S or R	pos./neg.	synergism

From South Africa we have the first report of an ESBL in *Haemophilus* spp. (10,17).

Besides, it is important to detect other types of resistance in Haemophilus:

B) Chloramphenicol
The diffusion method should be able to detect chloramphenicol resistance of Haemophilus. The most common type of **chloramphenicol resistance** of haemophilus is the production of a plasmidic acetyl transferase (CAT) that inactivates chloramphenicol. CAT can be detected using a "clover-leaf" technique, but when properly used the diffusion method is also able to detect **CAT producing strains**. The diffusion method using a break-point of **2 µg/ml** clearly separates chloramphenicol-susceptible from resistant strains.

C) Fluoroquinolones)
In Spain (4) ciprofloxacin-resistant *H .influenzae* have been isolated from patients with cystic fibrosis. Vigilance for quinolone-resistant H.influenzae should be maintained in patients with chronic infections treated with quinolones. Ciprofloxacin resistant *H. influenzae* have also been isolated in the UK (11), South Africa (12) and Spain (14).
The use of Nalidixan (R: < 25 mm) and Ciprofloxacin 0.5 µg Neo-Sensitabs with interpretations: S: ≥ 18 mm (MIC ≤ 0.12 µg/ml), I: 17-15 mm, R: ≤ 14 mm (MIC ≥ 1 µg/ml) is recommended in such cases. Current CLSI MIC breakpoints, will not always detect resistance.
In a long term care facility in New York, 36 % levofloxacin resistance was found among *H. influenzae* isolates in 2001 (9).

For the detection of decreased susceptibility to fluoroquinolones, due to changes in gyrA and ParC, the following breakpoints should be used (14):

			Zone diameter in mm			Break-points MIC µg/ml	
NEO-SENSITABS	**POTENCY**	**CODE**	**S**	**I**	**R**	**S**	**R**
Nalidixan	130 µg	NALID	-	-	< 25	-	≥ 16
Ciprofloxacin	0.5 µg	CIP.L	≥ 18	17-15	≤ 14	≤ 0.12	≥ 1
Ciprofloxacin	10 µg	CIP10	≥ 32	-	-	≤ 0.12	-
Levofloxacin	5 µg	LEVOF	≥ 30	-	-	≤ 0.25	-
Gatifloxacin	5 µg	GATIF	≥ 30	-	-	≤ 0.25	-
Moxifloxacin	5 µg	MOXIF	≥ 30	-	-	≤ 0.25	-
Ofloxacin	10 µg	OFLOX	≥ 30	-	-	≤ 0.25	-

D) Telithromycin
According to Bogdanovich et al (18) *H. influenzae* strains with telithromycin MIC's ≥ 0.5 µg/ml (zone ≤ 25 mm) have efflux present (resistance mechanism). Antimicrobial therapy may be ineffective.

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15.1.1 Quality Control Limits for *Haemophilus influenzae* ATCC 49247

HTM-agar, Inoculum: McFarland 0.5, Incubation at 35 °C in 5-7 % CO_2

NEO-SENSITABS	POTENCY	CODE	Zone diameter in mm	MIC µg/ml	
Amoxycillin+Clavulanate	30+15 µg	AM+CL	20-26	4/2-8/4	
Ampicillin	**2.5 µg**	AMP.L	10-14	2-8	
Ampicillin	**33 µg**	AMP33	22-27	2-8	
Azithromycin	30 µg	AZITR	15-22	1-4	
Ceftobiprole (BAL 9141)	30 µg	-----	28-36	0.25-0.5	
Cefaclor	30 µg	CCLOR	13-18	128	
Cefotaxime	30 µg	CFTAX	34-42	0.12-0.5	
Ceftazidime	30 µg	CEZDI	29-36	0.12-1	
Ceftriaxone	30 µg	CETRX	32-39	0.06-0.25	
Cefuroxime	60 µg	CEFUR	20-26	16	
Cephalothin	66 µg	CLOTN	20-25	32	
Chloramphenicol	**10 µg**	CLR10	27-34	0.25-1	
Chloramphenicol	**60 µg**	CLR60	36-43	0.25-1	
Ciprofloxacin	10 µg	CIP10	34-42	0.004-0.03	
Clarithromycin	30 µg	CLARI	12-18	4-16	
Doripenem	10 µg	DORIP	21-31	0.12	
Erythromycin	78 µg	ERYTR	21-27	8	
Faropenem			15-22	-	
Gatifloxacin	5 µg	GATIF	33-41	0.008-0.016	
Iclaprim			24-33	-	
Levofloxacin	5 µg	LEVOF	32-40	0.016	
Moxifloxacin	5 µg	MOXIF	31-39	0.008-0.03	
Nalidixan	130 µg	NALID	36-44	1	
Quinupristin/Dalfopristin	15 µg	SYN15	15-21	4	
Telithromycin	15 µg	TEL15	17-23	2	
Tetracyclines	**10 µg**	TET10	9-14	8-16	
Tetracyclines	**80 µg**	TET80	17-23	8-16	
Tigecycline	15 µg	TIG15	23-31	0.12-0.25	
Trimethoprim+Sulfa	5.2+240 µg	TR+SU	30-41	0.03-0.6	(as trim)

Note: These quality control limits apply only to the tests conducted with *H. influenzae* ATCC 49247 using Haemophilus Test Medium.

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15.2 Susceptibility Testing of Gonococci

The recommended agar medium for testing gonococci is Gonococcus Agar Base, to which a 1% of a defined growth supplement is added after autoclaving.
The use of a cysteine - free growth supplement is not required for diffusion testing.

Procedure (1)
Use colonies taken directly from an overnight culture (chocolate agar) and make a suspension in broth or 0.9% saline equivalent to the 0.5 McFarland standard. Use the suspension for plate inoculation within 15 min.

Not more than 9 Neo-Sensitabs should be placed on a 150 mm agar plate or 4 Neo-Sensitabs onto a 90-100 mm plate.

The plates are incubated at 35°C with 5-7% CO2 for 20 to 24 hours and the inhibition zones measured to the nearest millimeter.

The susceptibility of the strains tested is determined according to the interpretation table below.

Neisseria gonorrhoeae
GC Agar Base with Supplements, Inoculum: McFarland 0.5
Incubation in 5-7 % CO_2, Break-points according to CLSI (M2-A8)

	NEO-SENSITABS	POTENCY	CODE	Zone diameter in mm S	I	R	Break-points MIC µg/ml S	R
	Azithromycin	30 µg	AZITR	≥ 26	-	≤ 25	≤ 1	≥ 2
a)	Cefepime	30 µg	CFEPM	≥ 32	-	-	≤ 0.5	-
	Cefixime	30 µg	CFFIX	≥ 32	-	-	≤ 0.25	-
a)	Cefotaxime	30 µg	CFTAX	≥ 32	-	-	≤ 0.5	-
	Cefotetan	30 µg	CFTTN	≥ 26	25-21	≥ 20	≤ 2	≥ 8
	Cefoxitin	60 µg	CFOXT	≥ 32	31-27	≤ 26	≤ 2	≥ 8
a)	Cefpodoxime	30 µg	CFPOX	≥ 32	-	-	≤ 0.5	-
a)	Ceftazidime	30 µg	CEZDI	≥ 32	-	-	≤ 0.5	-
a)	Ceftriaxone	30 µg	CETRX	≥ 32	-	-	≤ 0.25	-
	Cefuroxime	60 µg	CEFUR	≥ 32	31-27	≤ 26	≤ 1	≥ 4
	Chloramphenicol	60 µg	CLR60	≥ 30	29-27	≤ 26	≤ 4	≥ 8
b)	Ciprofloxacin	10 µg	CIP10	≥ 41	40-28	≤ 27	≤ 0.06	≥ 1
b)	Ciprofloxacin	0.5 µg	CIP.L	≥ 28	27-15	≤ 14	≤ 0.06	≥ 1
	Doxycycline	80 µg	DOXYC	≥ 36	35-27	≤ 26	≤ 0.25	≥ 2
	Erythromycin	78 µg	ERYTR	≥ 30	-	≤ 29	≤ 1	≥ 2
	Levofloxacin	5 µg	LEVOF	≥ 32	31-27	≤ 26	≤ 0.25	≥ 1
d)	Nalidixan (quino)	130 µg	NALID	-	-	< 28	-	-
	Ofloxacin	10 µg	OFLOX	≥ 32	31-25	≤ 24	≤ 0.25	≥ 2
	Oxacillin (screening penicillin)	**1 µg**	OXA.1	≥ 12	-	-	≤ 0.06 (pen)	-
	Oxacillin (screening penicillin)	**5 µg**	OXA.5	≥ 18	-	-	≤ 0.06 (pen)	
c)	Penicillin Low	5 µg	PEN.L	≥ 44	43-26	≤ 25	≤ 0.06	≥ 2
	Spectinomycin	200 µg	SPECT	≥ 23	22-20	≤ 19	≤ 32	≥ 128
e)	Tetracyclines	80 µg	TET80	≥ 42	41-34	≤ 33	≤ 0.25	≥ 2
e)	Tetracyclines	10 µg	TET10	≥ 32	31-26	≤ 25	≤ 0.25	≥ 2
	Trimethoprim+Sulfa	5.2+240 µg	TR+SU	≥ 32	31-27	≤ 26	≤ 0.5 (9.5)	≥ 2 (38)

a) The CLSI has not yet defined other categories than "S" due to the current absence of resistant strains.

b) Ciprofloxacin-resistant gonococci should be presumed to be resistant to other quinolones: ofloxacin, moxifloxacin, pefloxacin, norfloxacin and gatifloxacin (2).

c) A positive beta-lactamase test predicts resistance to penicillin, ampicillin, amoxycillin, ticarcillin and piperacillin.

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d) Nalidixan is useful to detect strains with reduced susceptibility to quinolones.
If the zone is < 28 mm, control MIC's of CIPRO, OFLOX etc. Two strains of gonococci NALI-susceptible and CIPRO-resistant were recently detected in the UK (7). Test both NALI and CIPRO.

e) Gonococci with zonediameters ≤ 26 mm (Tetracyclines 80 µg Neo-S) or ≤ 18 mm
(Tetracyclines 10 µg Neo-S) usually indicate a plasmid mediated tetracycline resistant gonococcus (TRNG).

When testing the susceptibility of gonococci towards penicillin, it should be possible to detect 2 types of resistance:

1) Beta-lactamase producing strains (plasmid resistance by a constitutive beta-lactamase TEM-1). Beta-lactamase producing strains of gonococci may be detected a) with a rapid **beta-lactamase test** e.g. Beta-lactamase (acido) Diagnostic Tablets (ROSCO code 45521), or b) using **Amoxycillin+Clavulanate** Neo-Sensitabs compared to Amoxycillin alone. Synergism (larger zone with Amox+Clav) will be seen in the presence of a beta-lactamase.

2) Chromosomal resistance (alterations of PBP 1 and PBP 2 or permeability reduction). Chromosomally mediated resistance in *N. gonorrhoeae* may represent a continuum in the evolution of the resistance of the gonococcus to various antimicrobial agents. In Denmark and Sweden approx.25% of the gonococci show MIC's of 0.25-1 µg/ml towards penicillin and 5% are totally resistant to penicillin with MIC's > 1 µg/ ml. The strains showing decreased sensitivity to penicillin show MIC's > 0.1 µg/ml and are detected using the current diffusion technique with **Penicillin Low** Neo-Sensitabs.

   Emergence in Japan of beta-lactamase negative strains, resistant to penicillin (MIC 2-8 µg/ml) and with decreased susceptibility to cefixime (MIC ≥ 0.5 µg/ml) and ceftriaxone (MIC ≥ 0.125 µg/ml). These strains had a mosaic PBP2 composed of fragments of PBP2 from *N. cinerea* and *N. perflava* (7, 8).

   According to Hoff (3) and confirmed by our own investigations, Oxacillin 1 µg - Neo-Sensitabs is useful to detect beta-lactamase negative gonococci with **decreased susceptibility to penicillin** (chromosomal resistance), as the case is with pneumococci (S: ≥ 12 mm, R: no zone). Oxacillin 5 µg Neo-Sensitabs may be used for the same purpose.

   Strains of **gonococci** with decreased susceptibility towards **ciprofloxacin** have been isolated from patients, who did not respond to ciprofloxacin treatment (4,6). CDC has proposed criteria for the interpretation of susceptibilities of gonococci to the quinolones (5). The use of a disk with lower content of antimicrobial is recommended for detecting these strains. We recommend the use of Ciprofloxacin 0.5 µg Neo-Sensitabs (see table).

15.2.1 Quality Control Limits for *N. gonorrhoeae* ATCC 49226

G C Agar Base with Supplements, Inoculum: McFarland 0.5, Incubation at 35 °C in 5-7 % CO_2

NEO-SENSITABS	POTENCY	CODE	Zone diameter in mm	MIC µg/ml
Azithromycin	30 µg	AZITR	29-35	-
Ceftriaxone	30 µg	CETRX	41-53	0.004-0.016
Cefuroxime	60 µg	CEFUR	32-38	0.25-1
Ciprofloxacin	0.5 µg	CIP.L	34-42	0.001-0.008
Gatifloxacin	5 µg	GATIF	45-56	0.004-0.008
Penicillin Low	5 µg	PEN.L	23-31	0.25-1
Spectinomycin	200 µg	SPECT	27-33	8-32
Tetracyclines	10 µg	TET10	22-28	0.25-1
Tigecycline	15 µg	TIG15	30-40	-

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15.3 Susceptibility Testing of Meningococci

The recommended agar medium is Mueller-Hinton Agar with 5% Blood added.
Use colonies taken directly from an overnight culture and make a suspension in broth or 0.9% saline equivalent to the 0.5 McFarland standard.
Use the suspension for plate inoculation within 15 min.
Not more than 9 Neo-Sensitabs should be placed on a 150 mm agar plate or 4 Neo-Sensitabs onto a 90-100 mm plate.
The plates are incubated at 35°C with 5-7% CO_2 for 20 to 24 hours and the inhibition zones measured to the nearest millimetre.
The susceptibility of the strains tested is determined according to the interpretation table below.
The MIC breakpoints, when available, are according to recommendations from CLSI (formerly NCCLS) (8).
Interpretative criteria are based upon population distributions of MIC's of various agents, published data on pharmacokinetics and clinical experience in combination with knowledge from different national guidelines.

Meningococci
Mueller-Hinton + 5 % Blood
Inoculum: McFarland 0.5 Incubation in 5-7 % CO_2
Break-points according to CLSI

				Zone diameter in mm			Break-points MIC µg/ml	
	NEO-SENSITABS	POTENCY	CODE	S	I	R	S	R
	Ampicillin	2.5 µg	AMP.L	≥ 28	27-24	≥ 23	≤ 0.12	≥ 2
c)	Azithromycin	30 µg	AZITR	≥ 20	-	≤ 19	≤ 2	-
	Cefotaxime	30 µg	CFTAX	≥ 36	-	≤ 35	≤ 0.12	-
	Ceftriaxone	30 µg	CETRX	≥ 36	-	≤ 35	≤ 0.12	-
	Chloramphenicol	60 µg	CLR60	≥ 32	31-27	≤ 26	≤ 2	≥ 8
	Chloramphenicol	10 µg	CLR10	≥ 20	19-17	≤ 16	≤ 2	≥ 8
c)	Ciprofloxacin	10 µg	CIP10	≥ 36	35-32	≤ 31	≤ 0.03	≥ 0.12
c)	Doxycycline *	80 µg	DOXYC	≥ 30	29-27	≤ 26	≤ 2	≥ 8
c)	Levofloxacin	5 µg	LEVOF	≥ 35	34-33	≤ 32	≤ 0.03	≥ 0.12
	Meropenem	10 µg	MEROP	≥ 30	-	≤ 29	≤ 0.25	-
c)	Minocycline	80 µg	MINOC	≥ 28	-	≤ 27	≤ 2	-
b)	Nalidixan (screen quino)	130 µg	NALID	-	-	< 28	-	-
c)	Ofloxacin *	10 µg	OFLOX	≥ 36	35-32	≤ 31	≤ 0.03	≥ 0.12
a)	Oxacillin (screen pen)	**1 µg**	OXA.1	≥ 10	-	no zone	≤ 0.06 (pen)	MIC
a)	Oxacillin (screen pen)	**5 µg**	OXA.5	≥ 16	-	≤ 15	≤ 0.06 (pen)	MIC
	Penicillin Low	5 µg	PEN.L	≥ 26	25-23	≤ 22	≤ 0.06	≥ 0.5
c)	Rifampicin	30 µg	RIFAM	≥ 30	-	≤ 29	≤ 0.5	≥ 2
c)	Sulphonamides	240 µg	SULFA	≥ 28	-	≤ 27	≤ 16	≥ 32
c)	Trimethoprim+Sulfa	5.2+240 µg	TR+SU	≥ 36	35-32	≤ 31	≤ 0.12/2.3	≥ 0.5/9.5

* Breakpoints have not been established by CLSI (NCCLS).

a) Oxacillin 1 µg and Oxacillin 5 µg Neo-Sensitabs are useful to screen for beta-lactamase negative meningococci with decreased susceptibility to penicillin (chromosomal resistance). If the zone is < 10 mm (Oxacillin 1 µg) or ≤ 15 mm (Oxacillin 5 µg), perform an MIC test for penicillin.

b) Nalidixan is useful to screen for strains with reduced susceptibility to quinolones. If the zone is < 28 mm, control MIC's of OFLOX etc. Strains showing decreased susceptibility to ciprofloxacin have been reported in Spain (7).

c) Used for prophylaxis only (not treatment) .

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15.4 Susceptibility Testing of *Moraxella catarrhalis*

Zone diameter interpretative standards are as indicated in the table below:

Moraxella catarrhalis
Mueller-Hinton plain or 5 % blood, Inoculum: McFarland 0.5

	NEO-SENSITABS	POTENCY	CODE	Zone diameter in mm S	I	R	Break-points MIC µg/ml S	R
a)	Amoxycillin	30 µg	AMOXY	compare zone to AMC			-	beta-lactamase
	Amoxycillin+Clav.	30+15 µg	AM+CL	≥ 24	-	≤ 23	4/2	8/4
a)	Ampicillin	**33 µg**	AMP33	compare zone to AMC			-	beta-lactamase
a)	Ampicillin	**2.5 µg**	AMP.L	≥ 26	25-21	≤ 20	≤ 0.12	≥ 1
	Azithromycin	30 µg	AZITR	≥ 20	19-17	≤ 16	≤ 2	≥ 8
	Cefaclor	30 µg	CCLOR	≥ 20	19-17	≤ 16	≤ 8	≥ 32
	Cefotaxime	30 µg	CFTAX	≥ 30	-	≤ 29	≤ 2	-
	Ceftazidime	30 µg	CEZDI	≥ 30	-	≤ 29	≤ 2	-
	Ceftriaxone	30 µg	CETRX	≥ 30	-	≤ 29	≤ 2	-
	Cefuroxime	60 µg	CEFUR	≥ 28	27-24	≤ 23	≤ 4	≥ 16
	Cefuroxime (oral)	60 µg	CEFUR	≥ 32	31-27	≤ 26	≤ 1	≥ 4
	Chloramphenicol	60 µg	CLR60	≥ 32	31-27	≤ 26	≤ 2	≥ 8
	Chloramphenicol	10 µg	CLR10	≥ 20	19-17	≤ 16	≤ 2	≥ 8
	Ciprofloxacin	10 µg	CIP10	≥ 24	-	≤ 23	≤ 1	-
	Clarithromycin	30 µg	CLARI	≥ 20	19-17	≤ 16	≤ 2	≥ 8
	Clindamycin	25 µg	CLIND	≥ 28	27-24	≤ 23	≤ 0.5	≥ 4
	Doxycycline	80 µg	DOXYC	≥ 27	26-23	≤ 22	≤ 2	≥ 8
	Erythromycin	78 µg	ERYTR	≥ 28	27-21	≤ 20	≤ 0.5	≥ 8
	Gatifloxacin	5 µg	GATIF	≥ 30	29-27	≤ 26	≤ 0.25	≥ 0.5
	Gentamicin	40 µg	GEN40	≥ 28	27-24	≤ 23	≤ 2	≥ 8
	Imipenem	15 µg	IMIPM	≥ 26	25-21	≤ 20	≤ 1	≥ 4
	Levofloxacin	5 µg	LEVOF	≥ 22	-	≤ 21	≤ 2	-
	Meropenem	10 µg	MEROP	≥ 24	23-21	≤ 20	≤ 1	≥ 2
	Moxifloxacin	5 µg	MOXIF	≥ 30	29-27	≤ 26	≤ 0.25	≥ 0.5
	Ofloxacin	10 µg	OFLOX	≥ 24	-	≤ 22	≤ 1	-
a)	Penicillin Low	5 µg	PEN.L	≥ 32	31-23	≤ 22	≤ 0.06	≥ 2
	Rifampicin	30 µg	RIFAM	≥ 28	27-24	≤ 23	≤ 1	≥ 4
	Spiramycin	200 µg	SPIRA	≥ 26	25-21	≤ 20	≤ 2	≥ 8
	Tetracyclines	**80 µg**	TET80	≥ 27	26-23	≤ 22	≤ 2	≥ 8
	Tetracyclines	**10 µg**	TET10	≥ 20	19-17	≤ 16	≤ 2	≥ 8
	Trimethoprim+Sulfa	5.2+240 µg	TR+SU	≥ 32	31-27	≤ 26	≤ 0.5 (9.5)	≥ 2 (38)

a) Beta-lactamase-positive strains should be reported resistant to penicillin, ampicillin, amoxycillin, ticarcillin and piperacillin. These strains may be detected using Amoxycillin+Clavulanate Neo-Sensitabs compared to Amoxycillin Neo-Sensitabs or Ampicillin 33 µg Neo-Sensitabs. Synergism (>5 mm larger zone with Amoxycillin+Clavulanate) will be seen in the presence of a beta-lactamase (BRO-1, BRO-2).

The CLSI has not yet defined MIC breakpoints for *Moraxella catarrhalis*. Interpretative criteria are based upon population distributions of MIC's of various agents, published data on pharmacokinetics and clinical experience in combination with knowledge from different national guidelines.

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15.5 Susceptibility Testing of Pneumococci

The recommended medium is Mueller-Hinton agar supplemented with 5% defibrinated sheep blood (1).

Growth from an overnight (16-20 h) sheep blood agar plate is suspended in Mueller-Hinton broth or 0.9% saline to a density equivalent to the 0.5 McFarland standard. The suspension is used for plate inoculation within 15 min.

The plates are incubated at 35°C in an atmosphere of 5-7% CO_2 for 20 to 24 hours, the inhibition zones measured to the nearest millimeter and interpreted according to the table below.

1) Penicillin
Results with Oxacillin 1 µg must be reported as susceptibility (S) or resistance (I/R) to penicillin and not to oxacillin as such. Penicillin susceptible strains show MIC ≤ 0.06 µg/ml, intermediate or relatively resistant: MIC 0.12-1 µg/ml and penicillin resistant strains MIC ≥ 2 µg/ml.

Schrag et al. (12) document the emergence of pneumococci with very high level of resistance to penicillin (MIC ≥ 8 µg/ml) in the USA. It is associated with high (MIC ≥ 8 µg/ml) amoxicillin and cefotaxime MIC's.

2) Cephalosporins
Many strains of *S. pneumoniae* are only intermediate in susceptibility to penicillin and unfortunately some pneumococci are now resistant to cefotaxime (CFTAX) and ceftriaxone (CETRX) and they have been associated with clinical failures. As a consequence, clinical laboratories should consider screening selected isolates for susceptibility to CFTAX and CETRX as well as to penicillin. That is particularly important for blood and CSF isolates.

Studies have shown that a surrogate disk (tablet) containing ceftizoxime (CEZOX), can be used to predict CFTAX and CETRX susceptibility of S.pneumoniae (7). This surrogate tablet (Ceftizoxime Neo-Sensitabs) can be used together with Oxacillin 1 µg Neo-Sensitabs, another surrogate that is already in use to test for susceptibility to penicillin.

Antimicrobial resistance has clearly emerged as a very serious problem in the United States (3) and other parts of the world.

The emergence of pneumococci resistant to broad-spectrum cephalosporins has limited the choice of antibiotics for the treatment of pneumococcal meningitis (4).

It appears that the altered PBP's that reduce the susceptibility of *S. pneumoniae* to penicillin, also adversely influence the potencies of CFTAX, CETRX and cefpirome in vitro (higher MIC values than the susceptible strains). Ceftazidime has poor activity (5). Chiu et al. (22) observed an increasing ceftriaxone resistance in *S. pneumoniae* from Taiwan.

3) Macrolides/Telithromycin
Resistance to macrolides among *S. pneumoniae* has reached high levels in many countries. There are 2 main types of resistance mechanisms, one (MLS_B phenotype) leading to high level resistance and the other (M phenotype) resulting in a lower level of resistance. It is important to be able to detect such strains.

Besides the high level macrolides-lincosamides-streptogamin B (MLS) resistance, which may be constitutive or inducible (clindamycin zone distorted in the vicinity of erythromycin), Shortridge et al (6) detected a novel low level macrolide resistance with clindamycin susceptibility in 41% of the erythromycin resistant *S. pneumoniae* examined. These data suggest that macrolide resistant pneumococci (and streptococci) should not be assumed to be inducible resistant to clindamycin, without performing an induction test (10).

Concerning detection of inducible clindamycin resistance, see page 117: double tablet induction test for streptococci.

Faccone et al. (16) describe the emergence of an *S. pneumoniae* clinical isolate with high level telithromycin resistance (MIC 256 µg/ml) and simultaneous resistance to fluoroquinolones (MIC levofloxacin 64 µg/ml).

Rantala et al (18) observed in 26 (of 210 erythromycin resistant) erm-B positive isolates, showing heterogeneous resistance to telithromycin, manifested by the presence of colonies inside the inhibition zone. When cultured and tested, these cells showed stabler, homogeneous and high level resistance to telithromycin.

4) Fluoroquinolones
As a consequence of the increasing use of fluoroquinolones, resistance has now emerged to this group of compounds (11).

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Pérez-Trallero (11) mentions that using fluoroquinolones to treat a strain that had an exising, but unapparent, first step mutation in the gyr A gene, probably favoured the development of high level resistance to quinolones observed in the later isolates.

Current CLSI breakpoints for fluoroquinolones on *S. pneumoniae* define many isolates as susceptible, even though they harbour QRDR mutations. The proposed microbiological resistance breakpoints (13) is the MIC (zone) in which > 50 % of the isolates carry QRDR mutations (14, 19, 20, 21).

Microbiological resistance break-points for fluoroquinolones (13)

NEO-SENSITABS	POTENCY	CODE	Zone diameter in mm S	Zone diameter in mm R	Break-points MIC µg/ml S	Break-points MIC µg/ml R
Levofloxacin	5 µg	LEVOF	-	≤ 20	-	> 1
Moxifloxacin	5 µg	MOXIF	-	≤ 25	-	> 0.12
Gatifloxacin	5 µg	GATIF	-	≤ 24	-	> 0.25
Norfloxacin	10 µg	NORFL	-	≤ 12	-	> 8

Streptococcus pneumoniae
Mueller-Hinton + 5 % blood, Inoculum: McFarland 0.5
Incubation in 5-7 % CO_2, Break-points according to CLSI (M2-A8)

	NEO-SENSITABS	POTENCY	CODE	Zone diameter in mm S	Zone diameter in mm I	Zone diameter in mm R	Break-points MIC µg/ml S	Break-points MIC µg/ml R
	Azithromycin	30 µg	AZITR	≥ 22	21-19	≤ 18	≤ 0.5	≥ 2
c)	Ceftizoxime (cefotaxime, ceftriaxone, cefepime, cefpirome)	30 µg	CEZOX	≥ 30	29-26	≤ 25	≤ 0.5 (CFTAX etc.)	MIC
	Chloramphenicol	**60 µg**	CLR60	≥ 28	-	≤ 27	≤ 4	≥ 8
	Chloramphenicol	**10 µg**	CLR10	≥ 16	-	≤ 15	≤ 4	≥ 8
	Clarithromycin	30 µg	CLARI	≥ 24	23-21	≤ 20	≤ 0.25	≥ 1
	Clindamycin	25 µg	CLIND	≥ 28	27-24	≤ 23	≤ 0.25	≥ 1
	Doxycycline	80 µg	DOXYC	≥ 30	29-27	≤ 26	≤ 2	≥ 8
	Erythromycin	78 µg	ERYTR	≥ 28	27-24	≤ 23	≤ 0.25	≥ 1
h) *)	Fosfomycin	70+40 µg	FOSFO	≥ 24	-	≤ 23	≤ 8	-
	Gatifloxacin	5 µg	GATIF	≥ 21	20-18	≤ 17	≤ 1	≥ 4
d)	Imipenem	15 µg	IMIPM	≥ 30	29-27	≤ 26	≤ 0.12	≥ 1
	Levofloxacin	5 µg	LEVOF	≥ 18	17-15	≤ 14	≤ 2	≥ 8
	Linezolid	30 µg	LINEZ	≥ 21	-	-	≤ 2	-
d)	Meropenem	10 µg	MEROP	≥ 28	27-24	≤ 23	≤ 0.25	≥ 1
d)	Moxifloxacin	5 µg	MOXIF	≥ 18	17-15	≤ 14	≤ 1	≥ 4
g)	Norfloxacin	10 µg	NORFX	-	-	≤ 12	Reduced susceptibility to quinolones	
	Ofloxacin	10 µg	OFLOX	≥ 20	19-17	≤ 16	≤ 2	≥ 8
a)	Oxacillin (penicillin)	1 µg	OXA.1	≥ 20	≤ 19	≤ 19	≤ 0.06 (pen)	≥ 0.12 (I/R) (pen)
	Quinupristin/Dalfopristin	15 µg	SYN15	≥ 19	18-16	≤ 15	≤ 1	≥ 4
e)	Rifampicin	30 µg	RIFAM	≥ 28	27-24	≤ 23	≤ 1	≥ 4
	Teicoplanin	60 µg	TEICO	≥ 18	-	-	≤ 1	-
	Telithromycin	15 µg	TEL15	≥ 19	18-16	≤ 15	≤ 1	≥ 4
	Tetracyclines	**80 µg**	TET80	≥ 30	29-27	≤ 26	≤ 2	≥ 8
	Tetracyclines	**10 µg**	TET10	≥ 20	19-17	≤ 16	≤ 2	≥ 8
	Tigecycline	15 µg	TIG15	≥ 19	-	-	≤ 0.25	
	Trimethoprim+Sulfa	5.2+240 µg	TR+SU	≥ 32	31-27	≤ 26	≤ 0.5/9.5	≥ 4/76
b)	Vancomycin	5 µg	VAN.5	≥ 17	-	-	≤ 1	-

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	NEO-SENSITABS	POTENCY	CODE	Zone diameter in mm S	I	R	Break-points MIC µg/ml S	R
				f) non-meningeal criteria (9)				
f)	Amoxycillin	30 µg	AMOXY	≥ 26	25-21	≤ 20	≤ 2	≥ 8
f)	Amoxycillin+Clav.	30+15 µg	AM+CL	≥ 26	25-21	≤ 20	≤ 2/1	≥ 8/4
f)	Cefepime	30 µg	CFEPM	≥ 28	27-24	≤ 23	≤ 1	≥ 4
f)	Cefotaxime	30 µg	CFTAX	≥ 28	27-24	≤ 23	≤ 1	≥ 4
f)	Cefpodoxime	30 µg	CFPOX	≥ 32	31-27	≤ 26	≤ 0.5	≥ 2
f)	Ceftriaxone	30 µg	CETRX	≥ 28	27-24	≤ 23	≤ 1	≥ 4
f)	Cefuroxime (oral)	60 µg	CEFUR	≥ 30	29-27	≤ 26	≤ 1	≥ 4

Note:
Penicillin, cefotaxime (or ceftriaxone) and meropenem should be tested by a reliable MIC method and reported routinely with **CSF isolates** of *S. pneumoniae.*

Comment: For cefotaxime, use of interpretation criteria for non-meningitis requires doses appropriate for serious pneumococcal infections; e.g. at least 1 g (adults) or 50 mg/kg (children) every 8 hours or more frequently.

*) Tentative (17).

a) Isolates with Oxacillin 1 µg zone ≥ 20 mm, are susceptible (MIC ≤ 0.06 µg/ml) to penicillin. A penicillin MIC as well as a cefotaxime/ceftriaxone MIC should be determined on isolates of *S. pneumoniae* with Oxacillin 1 µg zone sizes ≤ 19 mm (penicillin I/R). Penicillin resistant strains from the CSF, should be considered resistant to ampicillin, amoxycillin, amoxycillin + clavulanate and first/second generation cephalosporins. Strains susceptible to Oxacillin 1 µg (penicillin) can be considered susceptible to ampicillin, amoxycillin, amoxycillin+clavulanate, cefaclor, cefepime, cefixime, cefotaxime, cefpirome, ceftibuten, ceftriaxone, cefuroxime, cefpodoxime, ceftizoxime and imipenem for approved indications and these agents need not be tested.

b) The CLSI has not yet defined other categories than "S" due to the absence of vancomycin resistant strains. If strains showing smaller zones are observed, they should be submitted to a Reference Laboratory.

c) According to the CLSI for CSF isolates, cefotaxime and ceftriaxone should not be tested by the diffusion method. Ceftizoxime Neo-Sensitabs is used to predict the susceptibility of *S. pneumoniae* to cefotaxime (CFTAX) and ceftriaxone (CETRX). Strains with zones ≥ 30 mm are predictably susceptible to CFTAX and CETRX. Strains with zone ≤ 29 mm should be tested by an MIC method (7). (S ≤ 0.5 µg/ml, R ≥ 2 µg/ml).

d) Break-points not yet established by the CLSI.

e) If reported, laboratories should indicate that rifampicin should not be used alone for therapy.

f) For use against *S. pneumoniae* in acute otitis media, acute sinusitis and comunity acquired pneumonia (non-meningeal interpretative criteria). Breakpoints have been based primarily on pharmacokinetic and pharmaco-dynamic considerations (NCCLS 1998, 2001). Amoxycillin results are valid for Penicillin G and ampicillin.

g) Norfloxacin is used to screen for fluoroquinolone resistance. Isolates with a zone < 12 mm should be subjected to MIC test. Report results as fluoroquinolone S or R.

h) Fosfomycin to be used in combination with ceftriaxone or vancomycin (17).

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15.5.1 Quality Control Limits for *S. pneumoniae* ATCC 49619

Quality Control Limits for *S. pneumoniae* ATCC 49619
MH + 5 % blood, Inoculum: McFarland 0.5
Incubation in 5-7 % CO_2

	NEO-SENSITABS	POTENCY	CODE	Zone diameter in mm	MIC µg/ml
	Amoxycillin	30 µg	AMOXY	36-42	0.06/0.12
	Ampicillin	33 µg	AMP33	36-42	0.06/0.12
	Ceftobiprole (BAL 9141)	30 µg	-----	32-39	0.008-0.015
	Cefepime	30 µg	CFEPM	28-35	0.12
	Cefotaxime	30 µg	CFTAX	33-41	0.06
	Ceftizoxime	30 µg	CEZOX	28-34	0.25
	Ceftriaxone	30 µg	CETRX	33-40	0.06
	Cefuroxime	60 µg	CEFU	32-38	0.25/0.5
	Chloramphenicol	10 µg	CLR10	18-24	4
	Chloramphenicol	60 µg	CLR60	28-35	4
	Clindamycin	25 µg	CLIND	29-36	0.06/0.12
	Doripenem	10 µg	DORIP	30-38	0.06
	Doxycycline	80 µg	DOXYC	27-34	0.06
	Erythromycin	78 µg	ERYTR	28-35	0.06/0.12
	Faropenem			21-29	0.06
	Gatifloxacin	5 µg	GATIF	24-31	0.25
	Iclaprim			21-29	-
	Levofloxacin	5 µg	LEVOF	20-25	1
	Linezolid	30 µg	LINEZ	28-34	1
	Meropenem	10 µg	MEROP	30-37	0.12
	Moxifloxacin	5 µg	MOXIF	25-31	0.12
	Ofloxacin	10 µg	OFLOX	17-23	2
*	Oxacillin	1 µg	OXA.1	12-16	0.5 (pen)
	Penicillin Low	5 µg	PEN.L	22-28	0.5
	Quinupristin/Dalfopristin	15 µg	SYN15	19-24	0.5
	Telavancin	30 µg	-----	17-24	0.004-0.008
	Telithromycin	15 µg	TEL15	27-33	0.008-0.016
	Tetracyclines	10 µg	TET10	20-26	0.25
	Tetracyclines	80 µg	TET80	28-35	0.25
	Tigecycline	15 µg	TIG15	23-29	0.03-0.06
	Trimethoprim+Sulfa	5.2+240 µg	TR+SU	32-38	0.25+4.75
	Vancomycin	5 µg	VAN.5	19-26	0.25

* Deterioration in Oxacillin paper disks content may result in false resistance when testing pneumococci. Therefore the CLSI recommends (8) testing Oxacillin 1µg paper disks against both *S. pneumoniae* ATCC 49619 (penicillin intermediate) and *Staph. aureus* ATCC 25923 (penicillin sensitive acceptable zone diameter 18-24 mm). This problem is nonexistent when using Oxacillin 1µg Neo-Sensitabs, due to its high stability.

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15.6 Susceptibility Testing of Beta-haemolytic and Viridans Streptococci

The recommended medium is Mueller-Hinton agar supplemented with 5% defibrinated sheep blood.

Procedure (1)
Growth from an overnight (18-20 h) sheep blood agar plate is suspended in Mueller-Hinton broth or 0.9% saline, to a density equivalent to the 0.5 McFarland standard. Plates must be inoculated within 15 minutes.

The current tablet diffusion procedure described for Neo-Sensitabs should be followed, but no more than 9 Neo-Sensitabs should be placed on a large (150 mm) plate or 4 Neo-Sensitabs on a 90-100 mm plate.

Incubate the plate at 35 °C ±2 degrees in an atmosphere of 5-7% CO_2 for 20-24 hours before reading the inhibition zones.
Zone diameter interpretative standards are described in the table below.

Special interpretation for beta-haemolytic streptococci with penicillins and cephalosporins (CLSI)

			Zone diameter in mm			Break-points MIC µg/ml	
NEO-SENSITABS	**POTENCY**	**CODE**	**S**	**I**	**R**	**S**	**R**
Amoxycillin	30 µg	AMOXY	≥ 28	-	≤ 27	≤ 0.25	-
Ampicillin	33 µg	AMP33	≥ 28	-	≤ 27	≤ 0.25	-
Cefepime	30 µg	CFEPM	≥ 26	-	-	≤ 0.5	-
Cefotaxime	30 µg	CFTAX	≥ 26	-	-	≤ 0.5	-
Ceftriaxone	30 µg	CETRX	≥ 26	-	-	≤ 0.5	-
Penicillin Low	5 µg	PEN.L	≥ 22	-	-	≤ 0.12	-

Note: The beta-haemolytic group includes the large colony forming pyogenic strains of streptococci with group A (*S. pyogenes*), C, G antigens and strains with group B (S agalactiae) antigen.

Penicillin resistant strains of Group A and Group B streptococci have not yet been recognized. Any strains found to be intermediate or resistant should be referred to a Reference Laboratory for confirmation (NCCLS 2000).

Group B streptococci are susceptible to penicillin, ampicillin and cefazolin, but may be resistant to clindamycin and/or erythromycin. When a group B streptococci is isolated from a pregnant woman with severe penicillin allergy, clindamycin and erythromycin should be tested and reported. (See Double-tablet induction test (D Zone-test) page 117).

CLSI recommend disk diffusion zone diameter interpretive standards for Ampicillin (Amoxycillin) and Penicillin against Beta-haemolytic Streptococci. Pen Low Neo-Sensitabs: S ≥ 22 mm, Ampicillin 33 and Amoxycillin Neo-Sensitabs: S ≥ 28 mm. Strains showing zones less than 22 mm (Pen Low) or less than 28 mm (Ampi 33, Amox) should be sent to a Reference Laboratory (1).

CLSI recommend specific zone diameter interpretative criteria for Cefotaxime, Ceftriaxone and Cefepime against Beta Haemolytic Streptococci. Cefotaxime Neo-Sensitabs: S ≥ 26 mm, (MIC ≤ 0.5 µg/ml), Ceftriaxone Neo-Sensitabs: S: ≥ 26 mm (MIC ≤ 0.5 µg/ml) and Cefepime Neo-Sensitabs: S: ≥ 26 mm (MIC ≤ 0.5 µg/ml). Strains showing zones less than 26 mm with any of the 3 cefalosporins should be sent to a Reference Laboratory (1).

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Streptococci (except *S. pneumoniae*)
Mueller-Hinton 5 % blood, Inoculum: McFarland 0.5
Incubation in 5-7 % CO_2, Break-points according to CLSI (M100-S15)

	NEO-SENSITABS	POTENCY	CODE	Zone diameter in mm S	I	R	Break-points MIC µg/ml S	R
a)	Amoxycillin (viridans)	30 µg	AMOXY	≥ 30	29-21	≤ 20	≤ 0.25	≥ 8
a)	Ampicillin (viridans)	33 µg	AMP33	≥ 30	29-21	≤ 20	≤ 0.25	≥ 8
	Azithromycin	30 µg	AZITR	≥ 22	21-19	≤ 18	≤ 0.5	≥ 2
a)	Cefepime (viridans)	30 µg	CFEPM	≥ 26	25-23	≤ 22	≤ 1	≥ 4
a)	Cefotaxime (viridans)	30 µg	CFTAX	≥ 30	29-27	≤ 26	≤ 1	≥ 4
*	Cefpirome	30 µg	CFPIR	≥ 30	29-27	≤ 26	≤ 1	≥ 4
a)	Ceftriaxone (viridans)	30 µg	CETRX	≥ 28	27-24	≤ 23	≤ 1	≥ 4
	Chloramphenicol	**60 µg**	CLR60	≥ 28	27-21	≤ 20	≤ 4	≥ 16
	Chloramphenicol	**10 µg**	CLR10	≥ 16	15-11	≤ 10	≤ 4	≥ 16
	Clarithromycin	30 µg	CLARI	≥ 24	23-21	≤ 20	≤ 0.25	≥ 1
e)	Clindamycin	25 µg	CLIND	≥ 28	27-24	≤ 23	≤ 0.25	≥ 1
	Daptomycin (+Ca) 2+18 h prediffusion	30 µg	DAPCa	≥ 22	-	-	≤ 1	-
*	Doxycycline	80 µg	DOXYC	≥ 26	25-23	≤ 22	≤ 2	≥ 8
e)	Erythromycin	78 µg	ERYTR	≥ 28	27-24	≤ 23	≤ 0.25	≥ 1
	Gatifloxacin	5 µg	GATIF	≥ 21	20-18	≤ 17	≤ 1	≥ 4
*	Imipenem	15 µg	IMIPM	≥ 30	29-27	≤ 26	≤ 0.25	≥ 1
b)	Levofloxacin	5 µg	LEVOF	≥ 18	17-15	≤ 14	≤ 2	≥ 8
	Linezolid	30 µg	LINEZ	≥ 21	-	-	≤ 2	-
	Meropenem	10 µg	MEROP	≥ 28	-	-	≤ 0.5	-
	Moxifloxacin	5 µg	MOXIF	≥ 18	17-15	≤ 14	≤ 1	≥ 4
b)	Ofloxacin	10 µg	OFLOX	≥ 20	19-17	≤ 16	≤ 2	≥ 8
	Oxacillin (penicillin screening)	**1 µg**	OXA.1	≥ 14	≤ 13	≤ 13	≤ 012 (pen)	≥ 0.25 (I/R)
	Oxacillin (penicillin screening)	**5 µg**	OXA.5	≥ 20	≤ 19	≤ 19	≤ 012 (pen)	≥ 0.25 (I/R)
a)	Penicillin Low (viridans)	5 µg	PEN.L	≥ 26	25-13	≤ 12	≤ 0.12	≥ 4
	Quinupristin/Dalfopristin	15 µg	SYN15	≥ 19	18-16	≤ 15	≤ 1	≥ 4
d)	Rifampicin	30 µg	RIFAM	≥ 28	27-24	≤ 23	≤ 1	≥ 4
f)	Tigecycline	15 µg	TIG15	≥ 19	-	-	≤ 0.25	-
	Teicoplanin	60 µg	TEICO	≥ 18	-	-	-	-
g)	Telithromycin	15 µg	TEL15	≥ 19	18-16	≤ 15	≤ 1	≥ 4
	Tetracyclines	**80 µg**	TET80	≥ 26	25-23	≤ 22	≤ 2	≥ 8
	Tetracyclines	**10 µg**	TET10	≥ 18	17-15	≤ 14	≤ 2	≥ 8
	Trimethoprim+Sulfa	5.2+240 µg	TR+SU	≥ 32	31-27	≤ 26	≤ 0.5 (9.5)	≥ 4 (76)
c)	Vancomycin	5 µg	VAN.5	≥ 17	-	-	≤ 1	-

* Breakpoints have not been established by the CLSI.

Note: Viridans streptococci isolated from blood or CSF should be tested for penicillin or ampicillin susceptibility using an MIC method. (1)

Small colony-forming beta-haemolytic strains with group A, C, F or G antigens (S. anginosus, previously termed *S. milleri*) are considered part of the viridans group. The viridans group also includes: *S. mitis*, *S. oralis*, *S. sanguis*, *S. salivarius*, *S. intermedius*, *S. constellatus*, *S. mutans* and *S. bovis*.

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a) Strains susceptible to Penicillin Low can be considered susceptible to ampicillin, amoxycillin, amoxycillin+clavulanate, ampicillin+sulbactam, cefaclor, cefepime, cefotaxime, ceftriaxone, cefuroxime, cefpodoxime, ceftizoxime, and imipenem for approved indications, and need not be tested against these agents. Penicillin resistant strains from the CSF should be considered resistant to ampicillin, amoxycillin, amoxycillin+clavulanate and first/second generations cephalosporins.
The CLSI recommends only testing of beta-haemolytic streptococci.

b) The CLSI recommends only testing of beta-haemolytic streptococci against Ofloxacin/Levofloxacin.

c) The CLSI has not yet defined other categories than "S" due to the absence of vancomycin resistant strains. Strains yielding results suggestive of a "non susceptible" category should be submitted to a Reference Laboratory for further testing.

d) Rifampicin should not be used alone for chemotherapy.

e) If erythromycin-resistant β-hemolytic streptococci use D Zone-test to detect resistance phenotype.

f) Tentative FDA breakpoints, September 2005.

g) Macrolide resistant isolates (erm B) may show heterogeneous resistance to telithromycin (colony growth inside the inhibition zone). Report as resistant to telithromycin (12).

Interpretative criteria for streptococci are based on population distributions of various species, pharmacokinetics of the antimicrobials, previously published literature, and the clinical experience of certain members of the CLSI Subcommittee (2).

Oxacillin 1 µg Neo-Sensitabs is useful for screening for penicillin susceptibility in streptococci. Strains with inhibition zones ≥ 14 mm are susceptible to penicillin, while strains showing zones ≤ 13 mm (I/R) should be tested for penicillin susceptibility by an MIC method. Oxacillin 5 µg Neo-Sensitabs is also useful (SFM 2002).

Since 1983, there has been several reports disclosing high rates of penicillin resistant viridans streptococci, isolated from clinical significant infections. Penicillin resistance is due to alterations in the PBP's.

A study of 410 consecutive viridans streptococcal isolates from blood to 22 beta-lactams was performed in Barcelona (3). 33.6% were found resistant to penicillin (MIC 0.5-8 µg/ml). The most active drug against the resistant strains was imipenem followed by cefpirome, cefotaxime, ceftriaxone and cefepime. Rodriguez-Avial et al. (6) found 49 % erythromycin resistant and 46 % penicillin resistant viridans strains.

Decreased activity of erythromycin against *S. pyogenes* and other beta-haemolytic streptococci has been reported from several countries (4,5,7,8).

Horizontal gene transfer of fluoroquinolone resistance from *S. dysgalactiae* to *S. pyogenes* is desribed (10).
S. dysgalactiae may serve as a resistance gene pool for *S. pyogenes*.

Group B streptococci are susceptible to penicillin, ampicillin and cefazolin, but may be resistant to clindamycin and/or erythromycin. When a group B streptococcus is isolated from a pregnant woman with severe penicillin allergy, clindamycin and erythromycin should be tested and reported (1).

Double tablet induction test (D Zone-test)
The test is performed to detect the erythromycin resistance phenotypes. Erythromycin resistance is classified on the basis of the double-tablet test with Erythromycin and Clindamycin Neo-Sensitabs (Seppälä, (8)). The tablets are placed approx. 20 mm apart on Mueller-Hinton Agar supplemented with 5 % blood.

a) Resistance to both erythromycin and clindamycin indicates constitutive MLS_B cross-resistance (ErmB). Report resistance to erythromycin and clindamycin.
b) Blunting of the clindamycin zone proximal to Erythromycin Neo-Sensitabs indicates an inducible type of MLS_B (ErmA). The strain is reported as resistant to both erythromycin and clindamycin.
c) Susceptibility to clindamycin with no blunting of the zone indicates M-phenotype (efflux mechanism mefA). Report erythromycin resistant and clindamycin susceptible.
d) Erythomycin susceptibility (S) and Clindamycin I/R indicate the presence of LSA phenotype (lincosamide-streptogramin A) (9,11)

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15.7 Susceptibility Testing of Campylobacter

The recommended medium is Mueller-Hinton Agar with 5% blood added.
Use colonies taken directly from an overnight culture and make a suspension in broth or 0.9% saline equivalent to the 1.0 McFarland standard.
Use the suspension for plate inoculation within 15 min.
Not more than 9 Neo-Sensitabs should be placed on a 150 mm agar plate or 4 Neo-Sensitabs onto a 90-100 mm plate.
The plates are incubated at 36 °C in Campylobacter atmosphere + catalysator for 42 to 48 hours, or at 42 °C for 20-24 hours (same conditions), and the inhibition zones measured to the nearest mm.
The disk diffusion and the Etest have been demonstrated to be reliable and convenient methods (3,5). With the increased resistance it becomes important that susceptibility testing is performed rountinely by laboratories (5).
The susceptibility of the strains tested is determined according to the interpretation table below:

Interpretative zones for Campylobacter
Mueller-Hinton Agar + 5 % Blood. Inoculum: McFarland 1.0
Incubation: Campylobacter atmosphere + catalysator

			Zone diameter in mm			Break-points MIC µg/ml	
NEO-SENSITABS	**POTENCY**	**CODE**	**S**	**I**	**R**	**S**	**R**
Amoxycillin	30 µg	AMOXY	≥ 28	27-24	≤ 23	≤ 2	≥ 8
Amoxycillin+Clavulanate	30+15 µg	AM+CL	≥ 28	27-24	≤ 23	≤ 2	≥ 8
Ampicillin	33 µg	AMP33	≥ 28	27-24	≤ 23	≤ 2	≥ 8
Azithromycin	30 µg	AZITR	≥ 23	22-20	≤ 19	≤ 1	≥ 4
Cefotaxime	30 µg	CFTAX	≥ 28	27-24	≤ 23	≤ 4	≥ 16
Cephalothin (identification)	66 µg	CLOTN	≥ 18	-	< 16 (ID)	-	> 32
Chloramphenicol	60 µg	CLR60	≥ 28	27-24	≤ 23	≤ 4	≥ 16
Ciprofloxacin (8)	**10 µg**	CIP10	≥ 24	23-21	≤ 20	≤ 1	> 4
Ciprofloxacin (8)	**0.5 µg**	CIP.L	≥ 16	15-13	≤ 12	≤ 1	> 4
Clarithromycin	30 µg	CLARI	≥ 23	22-20	≤ 19	≤ 1	≥ 2
Clindamycin	25 µg	CLIND	≥ 28	27-24	≤ 23	≤ 1	≥ 2
Doxycycline	80 µg	DOXYC	≥ 28	27-24	≤ 23	≤ 2	≥ 8
Erythromycin (8)	78 µg	ERYTR	≥ 22	21-15	≤ 14	≤ 8	> 32
Furazolidone	50 µg	FURAZ	≥ 28	27-24	≤ 23	≤ 2	≥ 4
Gentamicin	40 µg	GEN40	≥ 28	27-24	≤ 23	≤ 2	> 4
Imipenem	15 µg	IMIPM	≥ 28	27-24	≤ 23	≤ 1	≥ 2
Levofloxacin	5 µg	LEVOF	≥ 23	22-20	≤ 19	≤ 1	> 2
Meropenem	10 µg	MEROP	≥ 28	27-24	≤ 23	≤ 1	≥ 2
Moxifloxacin	5 µg	MOXIF	≥ 26	25-23	≤ 22	≤ 0.5	> 1
Nalidixan (identification)	130 µg	NALID	≥ 18	-	< 16 (ID)	-	> 128
- (quinolones)			-	-	≤ 27	-	decreased suscept. to quinolones
Neomycin	120 µg	NEOMY	≥ 30	29-24	≤ 23	≤ 8	≥ 32
Nitrofurantoin	260 µg	NITRO	≥ 30	29-27	≤ 26	≤ 8	≥ 16
Norfloxacin	10 µg	NORFX	≥ 26	25-23	≤ 22	≤ 0.5	> 1
Ofloxacin	10 µg	OFLOX	≥ 26	25-23	≤ 22	≤ 0.5	> 1
Tetracyclines	80 µg	TET80	≥ 28	27-24	≤ 23	≤ 4	≥ 16
Trimethoprim+Sulfa	5.2+240 µg	TR+SU	≥ 28	27-24	≤ 23	≤ 2/38	≥ 8/152

(ID) = ID purposes

Please note:
Strains resistant to Nalidixan show a decreased sensitivity to the quinolones (CIPRO, NORFX, OFLOX etc.).

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15.8 Susceptibility Testing of *Vibrio cholerae*

The recommended medium is Mueller Hinton agar. Inoculum is prepared by the growth method or by direct colony suspension in 0.9% saline to a density equivalent to 0.5 Mc Farland standard. Incubate the plates at 35 °C in ambient air for 16-18 hours, before reading the inhibition zones.
Zone diameter interpretative standards with breakpoints according to CLSI are as follows:

				Zone diameter in mm			Break-points MIC µg/ml	
NEO-SENSITABS	**POTENCY**	**CODE**	**S**	**I**	**R**	**S**	**R**
a)	Ampicillin	33 µg	AMP33	≥ 20	19-17	≤ 16	≤ 8	≥ 32
	Chloramphenicol	60 µg	CLR60	≥ 25	24-21	≤ 20	≤ 8	≥ 32
b)	Ciprofloxacin	10 µg	CIP10	≥ 26	-	≤ 25	≤ 0.5	≥ 1
	Doxycycline	80 µg	DOXYC	≥ 20	19-17	≤ 16	≤ 4	≥ 16
b)	Erythromycin	78 µg	ERYTR	-	-	≤ 18	-	≥ 16
b) d)	Nalidixan	130 µg	NALID	-	-	< 25	-	> 8
	Sulfonamides	240 µg	SULFA	≥ 23	22-20	≤ 19	≤ 100	≥ 350
c)	Tetracyclines	**80 µg**	TET80	≥ 23	22-20	≤ 19	≤ 4	≥ 16
c)	Tetracyclines	**10 µg**	TET10	≥ 16	15-14	≤ 13	≤ 4	≥ 16
	Trimethoprim + Sulfa	5.2+240 µg	TR+SU	≥ 28	27-24	≤ 23	≤ 2 (38)	≥ 8 (152)

a) Results for ampicillin, are used to predict susceptibility to amoxycillin.

b) Not yet established by the CLSI.

c) Results for tetracycline can be used to predict susceptibility to doxycycline.

d) Strains resistant to nalidixan show a decreased susceptibility to quinolones (Ciprofloxacin MIC ≥ 0.12 mg/l).

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15.9 Susceptibility Testing of *Helicobacter pylori*

The recommended agar medium is Mueller-Hinton Agar + 10% blood.
Use colonies taken directly from a blood agar culture (incubated for 48 hours at 35-37°C under microaerophilic conditions). Control microscopically (with gram-staining) that there are only few cocoid cells in the culture.
Prepare a suspension in bouillon or 0.9% saline to an opacity equivalent to McFarland 3 or 4.
Not more than 9 Neo-Sensitabs should be placed on a 150 mm plate or 4 Neo-Sensitabs into a 90-100 mm plate.
The plates are incubated at 35°C in microaerophilic atmosphere for 72 hours.

***Helicobacter pylori* ***
Mueller-Hinton + 10 % Blood
Inoculum: 3-4 McFarland Incubation: microaerophilic

NEO-SENSITABS	POTENCY	CODE	Zone diameter in mm S	I	R	Break-points MIC µg/ml S	R
Amoxycillin	30 µg	AMOXY	≥ 32	31-27	≤ 26	≤ 0.5	≥ 2
Ampicillin	**33 µg**	AMP33	≥ 32	31-27	≤ 26	≤ 0.5	≥ 2
Ampicillin	**2.5 µg**	AMP.L	≥ 23	22-20	≤ 19	≤ 0.5	≥ 2
Azithromycin	30 µg	AZITR	≥ 30	29-24	≤ 23	≤ 0.25	≥ 1
Ciprofloxacin	**0.5 µg**	CIP.L	≥ 20	19-17	≤ 16	≤ 0.5	≥ 1
Clarithromycin	30 µg	CLARI	≥ 30	29-24	≤ 23	≤ 0.25	≥ 1
Doxycycline	80 µg	DOXYC	≥ 32	31-29	≤ 28	≤ 2	≥ 8
Erythromycin	78 µg	ERYTR	≥ 30	29-24	≤ 23	≤ 1	≥ 4
Furazolidone	50 µg	FURAZ	≥ 32	31-29	≤ 28	≤ 1	≥ 4
Levofloxacin	5 µg	LEVOF	≥ 26	-	< 26	< 1	≥ 1
Metronidazole	16 µg	MTR16	≥ 26	25-23	≤ 22	≤ 8	≥ 16
Tetracycline	10 µg	TET10	≥ 23	22-20	≤ 19	≤ 2	≥ 8

* MIC breakpoints not yet established by the CLSI.

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15.10 Susceptibility Testing of *S. maltophilia*, *B. cepacia,* and *Acinetobacter* spp.

The medium recommended is plain Mueller-Hinton Agar.
Use colonies taken directly from an overnight culture and make a suspension in 0.9% saline equivalent to the 0.5 McFarland standard. Use the suspension for plate inoculation within 15 min.
The plates are incubated in air at **30 °C** for 20-24 hours. *Acinetobacter* spp. at 35 °C. The CLSI Working Group recommends incubation at 35 °C (20-24 hours) for *S. maltophilia* and *B. cepacia.*
Incubation at higher temperatures (35-37°C) may result in false susceptibility results, particularly with aminoglycosides and polymyxins (7,9,14).
S. maltophilia show medium-dependent susceptibility. False susceptibility to beta-lactam antimicrobials is often found on Iso-sensitest Agar (8,14).
The susceptibility of the strains tested is determined according to the interpretation table below:

***Stenotrophomonas maltophilia* / *Burkholderia cepacia* * / *Acinetobacter* spp.**
Mueller-Hinton Agar plain. Inoculum Mcfarland 0.5
Incubation at 30 °C for 20-24 hours (*S. maltophilia*, *B. cepacia*)
Incubation at 35 °C for 16-18 hours (*Acinetobacter* spp.)

	NEO-SENSITABS	POTENCY	CODE	Zone diameter in mm S	I	R	Break-points MIC µg/ml S	R
	Amikacin (*Acinetobacter* spp.)	40 µg	AMIKA	≥ 20	19-17	≤ 16	≤ 16	≥ 32
	Ampicillin+Sulbactam (*Acinetobacter* spp.)	30+30 µg	AM+SU	≥ 20	19-17	≤ 16	≤ 8/4	≥ 32/16
	Aztreonam (*Acinetobacter* spp.)	30 µg	AZTRM	≥ 23	22-20	≤ 19	≤ 8	≥ 32
	Cefepime (*Acinetobacter* spp.)	30 µg	CFEPM	≥ 20	19-17	≤ 16	≤ 8	≥ 32
	Ceftazidime **	30 µg	CEZDI					
	Acinetobacter spp.			≥ 20	19-17	≤16	≤ 8	≥ 32
	B. cepacia			≥ 22	21-17	≤ 16	≤ 8	≥ 32
	Chloramphenicol	60 µg	CLR60	≥ 25	24-21	≤ 20	≤ 8	≥ 32
	Ciprofloxacin	10 µg	CIP10	≥ 26	25-21	≤ 20	≤ 1	≥ 4
a)	Colistin (*P. aeruginosa/Acinetobacter* spp.)	10 µg	CO.10	-	-	-	≤ 2	≥ 4
a)	2+18 h prediffusion			≥ 15	14-11	≤ 10	≤ 2	≥ 8
	Doxycycline	80 µg	DOXYC	≥ 26	25-23	≤ 22	≤ 4	≥ 16
	Acinetobacter spp.			≥ 22	21-19	≤ 18	≤ 4	≥ 16
	Gentamicin (*Acinetobacter* spp.)	40 µg	GEN40	≥ 23	22-20	≤ 19	≤ 1	≥ 4
b)	Imipenem (*B. cepacia*/*Acinetobacter* spp.)	15 µg	IMIPM	≥ 23	22-19	≤ 18	≤ 4	≥ 16
	Imipenem+EDTA	15+750µg	IM+ED	detection of metallo-ß-lactamases				
	Levofloxacin **	5 µg	LEVOF	≥ 23	22-20	≤ 19	≤ 2	≥ 8
b)	Meropenem ** (*B. cepacia*/*Acinetobacter* spp.)	10 µg	MEROP	≥ 23	22-19	≤ 18	≤ 4	≥ 16
	Minocycline **	80 µg	MINOC	≥ 26	25-23	≤ 22	≤ 4	≥ 16
	Acinetobacter spp.			≥ 22	21-19	≤ 18	≤ 4	≥ 16
	Moxifloxacin	5 µg	MOXIF	≥ 23	22-20	≤ 19	≤ 2	≥ 8
	Ofloxacin	10 µg	OFLOX	≥ 23	22-20	≤ 19	≤ 2	≥ 8
	Piperacillin (*B. cepacia*/*Acinetobacter* spp.)	100 µg	PIPRA	≥ 26	25-21	≤ 20	≤ 16	≥ 128
	Piperacillin+Tazo (*B. cepacia*/*Acinetobacter* spp.)	100+10µg	PI+TZ	≥ 26	25-21	≤ 20	≤ 16/4	≥ 128/4
	Polymyxins 150 (*S. maltophilia*/*Acinetobacter* spp.)	150 µg	CO150	≥ 22	-	≤ 21	≤ 4	≥ 8
	Rifampicin	30 µg	RIFAM	≥ 23	22-20	≤ 19	≤ 4	≥ 16

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NEO-SENSITABS	POTENCY	CODE	Zone diameter in mm S	I	R	Break-points MIC µg/ml S	R
Temocillin (*B. cepacia*)	30 µg	TEMOC	≥ 18	17-15	≤ 14	≤ 16	≥ 32
Tetracyclines	80 µg	TET80	≥ 26	25-23	≤ 22	≤ 4	≥ 16
Acinetobacter spp.			≥ 22	21-19	≤ 20	≤ 4	≥ 16
Ticarcillin+Clavulanate (*S. maltophilia*/*Acinetobacter* spp.)	75+15 µg	TI+CL	≥ 26	25-21	≤ 20	≤ 16/2	≥ 128/2
Tigecycline (*Acinetobacter* spp./ *Burkholderia* spp. (18))	15 µg	TIG15	≥ 16	-	-	≤ 2	-
Trimethoprim+Sulfa **	5.2+240 µg	TR+SU	≥ 30	29-24	≤ 23	≤ 2/38	≥ 8/152

* Interpretative zones are Tentative for 1 year. Breakpoints have not yet been established by the CLSI.

** Proposed MIC breakboints by the CLSI Working Group on *Stenotrophomonas* and *Burkholderia* (August 2003).

The Subcommittee for Antimicrobial Susceptibility Testing has established the Working Group for Susceptibility Testing of *S. maltophilia* and *B. cepacia*. CLSI recommends only minocycline, levofloxacin and trim-sulfa to be reported for *S. maltophilia* isolates and only ceftazidime, meropenem and minocycline to be reported for *B. cepacia* isolates (13).

a) Due to the high molecular weight of colistin, its diffusion on agar is very slow, resulting in small differences in inhibition zones between susceptible and resistant strains (Zones are tentative for one year).
Rosco Diagnostica has developed a prediffusion technique for colistin permitting a clear differentiation between susceptible and resistant strains (page 18).

   Place Colistin 10 µg Neo-Sensitabs on a non-inoculated MH plate and incubate at room temperature for 2 hours. Thereafter eliminate the tablet by knocking the place against the table and leave the plate at room temperature for further 18 hours overnight. Inoculate the plate and incubate overnight. Read the inhibition zones.

b) Resistant subpopulations (colonies) were grown within the zone of inhibition around imipenem and meropenem discs with *A. baumannii* strains in Greece (heteroresistance), showing MIC's of 8-32 µg/ml (imipenem) and meropenem MIC's of 4-16 µg/ml (15).

   Lesho et al (16) and Jones et al. (17) documented a greater potency and higher susceptibility rates for imipenem compared to meropenem, when *Acinetobacter* spp. are tested.
Reversed for *P. aeruginosa*, where in isolates from the U.S., meropenem showed a 2 to 4 fold greater potency than imipenem, and a percentage susceptibility advantage of 4-5 %.
Because of possible discords when testing carbapenems against non-fermenters, the carbapenem selected for therapy should also be tested by the clinical laboratory.

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16 Detection of Beta-Lactamases

16.1 Extended-Spectrum Beta-Lactamases (ESBL)

Screening and Confirmatory Tests for Extended-Spectrum Beta-Lactamases (ESBL)

Transferable plasmid-mediated beta-lactamases that produce resistance towards third generation cephalosporins and monobactams (e.g. aztreonam) have been described in strains of *Klebsiella pneumoniae*, *K. oxytoca*, *E. coli* and other Enterobacteriaceae. These enzymes are classified as extended-spectrum beta-lactamases (ESBL) and they have been implicated in clinical resistance to monobactams and broad-spectrum cephalosporins such as ceftazidime (CEZDI), cefotaxime (CFTAX), and ceftriaxone (CETRX).

Some ESBLs confer high-level resistance to these beta-lactams and are easily detected as resistant (or intermediate) by disk (tablet) diffusion testing. But the ESBL may provide low levels of resistance (MIC 1-2 µg/ml) to monobactams and third generation cephalosporins that can be easily overlooked by routine susceptibility methods and current interpretative criteria (1). These latter isolates may not reach current CLSI breakpoints for resistance, yet can be clinically resistant to beta-lactam therapy (2).

Since some ESBLs are more active on CEZDI, while others are more active on CFTAX, the choice of cephalosporins tested can also affect the ability of laboratories to detect resistant strains (3).
Most ESBLs are inhibited by clavulanic acid, tazobactam or sulbactam and can be readily detected by the double-disk (tablet) synergy test (4).

Double disk (tablet) synergy test

Inoculate a Mueller-Hinton plate as for susceptibility testing and apply Ceftriaxone (CETRX) Neo-Sensitabs, Cefotaxime (CFTAX) Neo-Sensitabs, Ceftazidime (CEZDI) Neo-Sensitabs, Cefepime (CFEPM) Neo-Sensitabs and Aztreonam (AZTRM) Neo-Sensitabs **at approximately 20 mm** (30 mm from tablet center to tablet center) from a tablet containing Amoxycillin+Clavulanate Neo-Sensitabs (AM+CL) using a dispenser. Incubate overnight at 35 °C.

Extension of the zone of inhibition (synergism) towards the tablet containing AM+CL, indicates the presence of an extended spectrum beta-lactamase (ESBL).

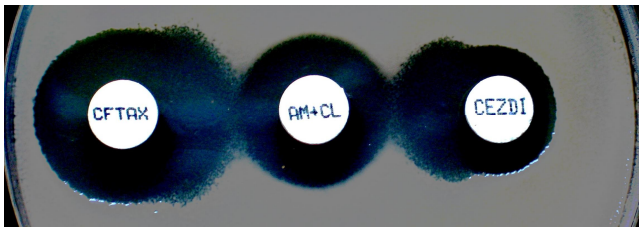

Plate 16.1-a. *Klebsiella pneumoniae* (ATCC 700603) producing extended-spectrum beta-lactamases (ESBL). Note the synergy between cefotaxime Neo-Sensitabs (CFTAX), ceftazidim Neo-Sensitabs (CEZDI) and Amoxycillin+Clavulanate Neo-Sensitabs (AM+CL).

Another possibility of screening for ESBL is the use of lower MIC break-points for ceftazidime and aztreonam. Livermore et al (5) showed that most ESBL producers were resistant to CEZDI at 2 µg/ml and AZTRM at 1 µg/ml. The corresponding zones with Neo-Sensitabs using McFarland 0.5 inoculum are 24 mm (CEZDI and CETRX) and 26 mm (AZTRM). As a consequence, *Klebsiella* spp, *E. coli* and *Salmonella* spp. showing zones < 24 mm with Ceftazidime, Cefepime and/or Ceftriaxone Neo-Sensitabs and/or < 26 mm with Aztreonam and/or Cefotaxime Neo-Sensitabs, should be suspected of ESBL production. The CLSI has adopted practically all the same MIC breakpoints.

Cefpodoxime may also be used in the screening of ESBL. Zones < 20 mm should be suspected of strains with ESBL production (18). Recently, the CLSI changed their Cefpodoxime screening breakpoints for ESBL from ≥ 2 to ≥ 8 µg/ml (18).

In a study comparing several ESBL screening methods, Vercauteren et al. (10) found that the double tablet synergy test using Neo-Sensitabs detected 96.9 % of ESBL producers while the E-test ESBL Screen detected 81.2 %.

De Gheldre et al showed that synergism between ceftazidime and cefepime with clavulanate (Neo-Sensitabs) was very useful to detect ESBL in *Enterobacter aerogenes* from Belgian hospitals (13).

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Confirmatory Tests for ESBL

The CLSI (9) recommends the use of Ceftazidime in combination with Clavulanic acid: Ceftazidime+Clavulanate Neo-Sensitabs, as a phenotypic confirmatory test for the presence of ESBL. Perform the antibiogram using Mueller Hinton Agar and McFarland 0.5 inoculum. Test both Ceftazidime+Clavulanate, Cefepime+Clavulanate and Ceftazidime/ Cefepime Neo-Sensitabs.
An increase in zone diameter of ≥ 5 mm for the combination Ceftazidime+Clavulanate or Cefepime+Clavulanate compared to Ceftazidime/ Cefepime alone is confirmatory of the presence of an ESBL.

Rodriguez-Villalobos et al. (20) and Fluit et al. (21) showed that the double disk (Neo-Sensitabs) synergy test has a higher sensitivity for the detection of ESBL than all combination disks (Oxoid, E-test).

Steward et al (12) showed that synergism between cefepime and clavulanate (Cefepime + Clavulanate Neo-Sensitabs) is very useful to detect ESBL in *Klebsiella pneumoniae*, differentiating strains producing ESBL (synergy between cefepime and clavulanate) from strains producing Amp C or hyperproducers of beta-lactamase.

Florijn et al (16) conclude that the use of ceftazidime, ceftriaxone and amoxycillin+clavulanate as Neo-Sensitabs is a cheap and reliable method for detection of *E. coli*, *Klebsiella* spp. and *P. mirabilis* isolates suspected of carrying ESBL. It performs better in a routine setting than the E-test, which often yields a result that cannot be interpreted.

Enterobacter, Serratia, *Morganella morganii*, Providencia, *Citrobacter freundii* and *Pseudomonas aeruginosa* produce chromosomally encoded inducible **Amp C** beta-lactamase. High level expression of Amp C may prevent the recognition of ESBL. Cefepime is practically not affected by Amp C and consequently Cefepime Neo-Sensitabs should be included as an ESBL screening agent when testing Enterobacter, Serratia ect. Synergism between Amox-Clav and Cefepime will indicate ESBL production (11,12,13,17,19). Strains with Cefepime zones < 24 mm should be suspected of ESBL production.

Recently Schwaber et al (32) found that the Vitek 2, Advanced Expert System identified the ESBL phenotype in only 62.5 % isolates of *Enterobacter* spp. and erroneously reported cephalosporin susceptibility in 28 %.

Cefepime+Clavulanate (and cefepime) Neo-Sensitabs should be used in the confirmatory tests for ESBL, because they are effective in detecting ESBL in strains of Klebsiella, *E. coli* etc. that may produce Amp C or are hyperproducers of beta-lactamase (31).

Pitout et al. (22) recommend the use of cefepime and piperacillin+tazobactam when testing against strains with high level expression of AmpC beta-lactamases (*E. cloacae*, *E. aerogenes*, *C. freundii*, *S. marcescens)*. Synergism between cefepime and tazobactam indicates presence of an ESBL.

Synergism between Ticarcillin + Clavulanate and aztreonam/ceftazidime/cefepime permit the detection of ESBL producing strains of *Ps. aeruginosa* (SFM 2001). These strains show currently no zone around Ceftazidime Neo-Sensitabs (14).

ESBLs can be obscured by the chromosomal AmpC cephalosporinase in *P. aeruginosa* (30). Cloxacillin 500 µg or Boronic acid Diatabs can be used to inhibit AmpC, for example by prediffusing (1 hour) one of these compounds on the agar before inoculation and before adding the antibiotic tablets (Neo-Sensitabs), placed on the same spots.

With *Klebsiella oxytoca*, synergism between Amoxycillin+Clavulanate (AM+CL) and Aztreonam or Ceftriaxone but not with ceftazidime indicates the presence of hyperproduction of K-1 chromosomal beta-lactamase (but **negative** for ESBL). Strains producing ESBL show synergism between AM+CL and ceftazidime (Use Ceftazidime+Clavulanate). The use of cefotaxime, ceftriaxone, cefepime, aztreonam with AM+CL may result in false positive results for ESBL in *Klebsiella oxytoca* (Vitek).

The emergence of ESBL in Salmonellae merits concern. They cause frequently neonatal meningitis in many developing countries and are often already resistant to ampicillin and chloramphenicol (7).
Karas et al (8) reports clinical failure due to ESBL, in spite of the organism being susceptible with disk diffusion and MIC test (CFTAX MIC 0.75 µg/ml). The double disk diffusion test indicated the presence of an ESBL, but the test was first performed when therapy with cefotaxime was stopped, due to treatment failure.
The laboratory report should indicate that ESBL-producing strains may be resistant clinically to all penicillins, cephalosporins and aztreonam (9).

For serious systemic infections, even if the isolate appears susceptible to Amoxycillin+Clavulanate, Ticarcillin+ Clavulanate or Piperacillin+Tazobactam, do not report it as susceptible, because resistant mutants may be selected during therapy.

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For Q.C. use *Klebsiella pneumoniae* ATCC 700603: zone of Ceftazidime+Clavulanate and Cefepime+Clavulanate is ≥ 5 mm larger than Ceftazidime/Cefepime Neo-Sensitabs (see chapter 16.2).

Detection of ESBLs using Neo-Sensitabs

ENTEROBACTERIACEAE
Strains showing cefotaxime and/or ceftazidime MICs ≥ 1 µg/ml, showing reduced susceptibility to amoxicillin + clavulanate should be tested further for the presence of ESBLs.

Procedure
Mueller Hinton agar plates are inoculated with the strain to be tested and Neo-Sensitabs applied onto the agar. Cefotaxime, Ceftazidime and Cefepime Neo-Sensitabs at a distance of 15-20 mm (edge to edge) from Amoxycillin + Clavulanate Neo-Sensitabs, or using their combinations: Cefotaxime + Clavulanate, Ceftazidime + Clavulanate, and Cefepime + Clavulanate Neo-Sensitabs.

Interpretation
A key hole or ghost zone between Amoxycillin + Clavulanate and any of Cefotaxime, Ceftazidime or Cefepime Neo-Sensitabs indicates the presence of an ESBL.
When using the combination disks, a ≥ 5 mm larger zone for any of the combinations compared to the corresponding single antimicrobial indicates the presence of an ESBL.
Cefpodoxime and Cefpodoxime + Clavulanate may be used for screening purposes.
Klebsiella oxytoca hyperproducing K-1 beta-lactamase may show a false positive result (potentiation of cofotaxime and /or cefepime). Only when the strain is resistant to ceftazidime and shows synergism between ceftazidime and clavulanate should it be reported as ESBL positive.

ESBL + AmpC beta-lactamases
In strains possessing both chromosomal/plasmidic AmpC beta-lactamases and ESBLs, Boronic acid can be used as inhibitor of the AmpC beta-lactamase (34, 35). For ex. 3 Boronic acid Diatabs are placed on the agar plate and eliminated after 1 hour at room temperature (prediffusion). The plate is now inoculated and the corresponding Neo-Sensitabs: Cefotaxime, Ceftazidime and Cefepime are placed where the Boronic acid disks were previously.
When using the combination disks, the same procedure with Boronic acid Diatabs is followed. An increase in the zones of inhibition of ≥ 3 mm (compared to the single drugs prediffused with boronic acid) indicates the presensce of an ESBL.
In most cases this procedure is not necessary, because with the use of Cefepime/Cefepime + Clavulanate (almost not affected by AmpC beta-lactamases) and Ceftazidime/Ceftazidime + Clavulanate it is possible to detect ESBLs in the presence of AmpC beta-lactamases.

NON-FERMENTERS
Here are particularly *P. aeruginosa* and *A. baumannii* that may possess several types of beta-lactamases. Non-fermenters showing reduced susceptibility to ceftazidime and/or cefepime and/or aztreonam should be tested for the presence of ESBLs.

Procedure
Apply Ceftazidime, Cefepime and Aztreonam Neo-Sensitabs. At a distance of approx. 15 mm (edge to edge) apply Ticarcillin + Clavulanate Neo-Sensitabs. Separately apply Ceftazidime + Clavulanate and Cefepime + Clavulanate Neo-Sensitabs.

Interpretation
A key-hole zone or ghost zone between Ticarcillin + Clavulanate and any of Ceftazidime, Cefepime or Aztreonam Neo-Sensitabs indicates the presence of an ESBL.
With the combination disks a ≥ 5 mm larger zone for Ceftazidime + Clavulanate and/or Cefepime + Clavulanate compared to the single antimicrobials indicates the presence of an ESBL.
The prediffusion procedure with Boronic acid may also be used (30) when ESBLs can be obscured by the chromosomal AmpC cephalosporinase in *P. aeruginosa*.

Detection of ESBLs in different strains

The presence of ESBLs may be masked by the overexpression of AmpC beta-lactamases or by the induction of AmpC beta-lactamase by clavulanate used in synergy tests. ESBLs may be confused with enzymes such as *K. oxytoca* chromosomal ß-lactamase (K1). Laboratory staff must be aware of the increasing array of different resistance mechanisms and phenotypes.

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1) *E. coli, Klebsiella* spp.*, Salmonella* spp.*, Proteus mirabilis, Shigella sonnei*:

ESBL	Synergism Ceftazidime+Clavulanate and/or Cefepime+Clavulanate. Cefoxitin S.
VEB-1 (ESBL) (25)	Synergism Ceftazidime+Clavulanate and/or Cefepime+Clavulanate. Synergism Imipenem and Ceftazidime and/or Cefoxitin and Ceftazidime.
AmpC plasmid (no ESBL)	No synergism Ceftazidime+Clavulanate and Cefepime+Clavulanate. Cefoxitin R, Ceftazidime R. Synergism Cefoxitin and Cloxacillin 500 µg, and/or Ceftazidime and Cloxacillin 500 µg.
DHA (Induc. plasmid AmpC)	Antagonism Clavulanate (AMC) and 3rd generation. Synergism Cefoxitin/Ceftazidime and Cloxacillin 500 µg.
DHA+ ESBL (33)	Antagonism Clavulanate (AMC) and 3rd gen. cephalosporins (DHA). Synergism Tazobactam (Piperacillin+Tazobactam) and Ceftazidime/Cefepime.
Amp C + ESBL	Synergism Cefepime+Clavulanate: ESBL (31). Cefoxitin R, Ceftazidime R. Synergism Cefoxitin or Ceftazidime and Cloxacillin 500 µg: AmpC.
DHA + ESBL	Synergism Ceftazidime + Clavulanate and/or Cefepime + Clavulanate: ESBL. Antagonism Clavulanate (AMC) and 3rd gen. cephalosporins: DHA/ACT-1.
ESBL + Metallo-beta-lactamases (24,28)	Synergism Aztreonam + Clavulanate (Amoxicillin+Clavulanate): ESBL. Synergism Imipenem+EDTA: metallo-beta-lactamases. Synergism Ceftazidime+DPA: metallo-beta-lactamases.
ESBL + 16S rRNA methylases (26)	Synergism Ceftazidime+Clavulanate and/or Cefepime+Clavulanate. Cefoxitin S. No zone with Amikacin, Gentamicin, Tobramycin Neo-Sensitabs.

2) High level K-1 (*Klebsiella oxytoca*):

(no ESBL)	No synergism Ceftazidime+Clavulanate. Ceftazidime S. Synergy is currently observed with third gen. cephalosporins and Clavulanate as well as with Cefepime+Clavulanate.
K-1 + ESBL (23)	Synergism Ceftazidime+Clavulanate. Ceftazidime I/R.

3a) *Enterobacter* spp.*, Serratia* spp.*, Providencia rettgeri, Citrobacter freundii*:

ESBL	Synergism Cefepime and Clavulanate (Amoxycyllin+Clavulanate) and/or Ceftazidime and Clavulanate.
ESBL + 16S rRNA methylases	Synergism Cefepime and Clavulanate and/or Ceftazidime +Clavulanate. No zone with Amicacin, Gentamicin, Tobramycin Neo-Sensitabs.
ESBL + Metallo-beta-lactamases	Synergism Aztreonam (or Cefepime)+Clavulanate: ESBL Synergism Imipenem + EDTA and/or Ceftazidime + DPA: Metallo-beta-lactamases.

3b) *Morganella morganii*

ESBL	Synergism Cefepime and Tazobactam (Piperazillin+Tazobactam). Synergism Sulbactam (Ampicillin+Sulbactam) and Ceftazidime or Cefotaxime.

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4) *Pseudomonas aeruginosa*:

ESBL	Ceftazidime no zone of inhibition. Ticarcillin resistant. Synergism Ceftazidime+Clavulanate and/or Cefepime+Clavulanate. Synergism Aztreonam/Ceftazidime or Cefepime with Ticarcillin+Clavulanate
VEB-1 (ESBL)	Synergism between Imipenem and Ceftazidime (or Cefepime) in the presence of Cloxacillin 500 µg. Synergism Ceftazidime+Clavulanate and/or Cefepime+Clavulanate.
ESBL + Metallo-beta-lactamases	Synergism Aztreonam (or Cefepime)+Clavulanate: ESBL Synergism Imipenem + EDTA and/or Ceftazidime + DPA:: Metallo-beta-lactamases.

5) *Acinetobacter* spp.

ESBL	Synergism Ceftazidime+Clavulanate and/or Cefepime+Clavulanate. Synergism Ceftazidime/Cefepime and Ticarcillin+Clavulanate.
VEB-1 (ESBL)	Synergism Cefepime and Ticarcillin+Clavulanate (distance 15 mm). Best at 30 °C in the presence of Cloxacillin 500 µg.

6) *Achromobacter xylosoxidans*

VEB-1 (ESBL)	Synergy between Ceftazidime and Clavulanate.

7) *Haemophilus influenzae*:

ESBL (27)	Compare Cefpodoxime and Cefpodoxime+Clavulanate. Zones: ≥ 5 mm larger with the combination.

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16.2 ESBL Quality Control

***Klebsiella pneumoniae* ATCC 700603**

NEO-SENSITABS	POTENCY	CODE	Zone diameter in mm	MIC µg/ml (15)
Aztreonam	30 µg	AZTRM	11-19	64
Cefepime	30 µg	CFEPM	21-26	1
Cefepime+Clavulanate	30+10 µg	CP+CL	28-33	≤ 0.12
Cefotaxime	30 µg	CFTAX	20-26	8
Cefpodoxime	30 µg	CFPOX	14-21	16
Ceftazidime	30 µg	CEZDI	11-19	32
Ceftazidime-Clavulanate	30+10 µg	CZ+CL	24-31	1
Ceftriaxone	30 µg	CETRX	16-24	16

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16.3 Inducible Cephalosporinases or AmpC Beta-lactamases

Inducible cephalosporinases or AmpC beta-lactamases are produced by *Enterobacter cloacae*, *E. aerogenes*, *Serratia marcescens*, *Citrobacter freundii*, *Hafnia alvei*, *Providencia stuartii* and *Morganelli morganii*, and they are inhibited by aztreonam, but not by clavulanic acid, sulbactam or tazobactam.
Resistant mutants with high beta-lactamase activity are present at a high frequency. As a result therapy with cephalosporins (except fourth generation agents) and monobactams may fail because of selection of such mutants.
The tablet approximation test is useful to demonstrate the presence of inducible cephalosporinases, during routine antibiogram testing.
Neo-Sensitabs containing an inducer, e.g. cefoxitin (or imipenem) and indicators such as piperacillin+tazobactam, cefotaxime or ceftazidime are placed approx. 20-25 mm apart center to center. A wider spacing (30 mm) may be preferable for e.g. *M. morganii* and *Providencia* spp.
Following overnight incubation at 35 °C in air, the presence of an inducible beta-lactamase is indicated by the blunting of the zone of inhibition around the indicator drug (piperacillin+tazobactam, cefotaxime/ceftazidime) adjacent to the inducer (cefoxitin/imipenem).

Dunne et al (1) have shown that the combination Imipenem and Piperacillin + Tazobactam has the highest sensitivity (97.1 %) followed by Imipenem and Ceftazidime (94.2 %).

The result should be reported as R (resistant) for penicillins (except temocillin), penicillin/inhibitor combinations, cephalosporins (except cefpirome and cefepime), cephamycins and monobactams, irrespective of the size of the inhibition zone.

Plate 16.3-a

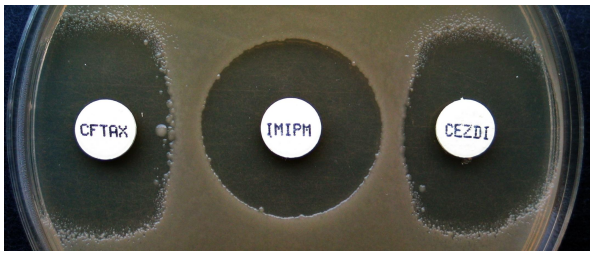

Plate 16.3-b

Demonstration of the presence of inducible beta-lactamases in *Enterobacter cloacae* (ATCC 13047). Note the flattened edges of Cefotaxime Neo-Sensitabs (CFTAX) and Ceftazidime Neo-Sensitabs (CEZDI) zones adjacent to Cefoxitin Neo-Sensitabs (CFOXT, Plate 16.3-a) and Imipenem Neo-Sensitabs (IMIPM, Plate 16.3-b), respectively.

References:

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16.3.1 Testing / Reporting of Susceptibility to Beta-lactams against Enterobacteriaceae and Non-fermenters

Use the table below for testing/reporting of susceptibility to beta-lactams against Enterobacteriaceae and non-fermenters causing serious infections when inducible beta-lactamases are present [a]:

	AMP	AM+CL	CEFUR	CFTAX	CETRX	CEZDI	CFEPM	CFOXT	AZTRM	IMIPM	MEROP	TI+CL	PI+TZ
E. aerogenes/*cloacae* *C. freundii* / *S. marcescens*	R	R	R	R	R	R	T	R	R	T	T	R	R
Prov. stuartii/rettgeri *Morg. morganii*	R	R	R	R	R	R	T	R	T	T	T	R	T
P. vulgaris/penneri	R	T	R	R	R	T	T	T	R	T	T	T	T
Klebsiella oxytoca [e]	R	T	R	R	R	T	T	T	R	T	T	T	T
Hafnia alvei	R	R	R	R	R	R	T	R	R	T	T	R	R
Enterobacteriaceae with ESBL (**no** inducible ß-lactamases)	R	T [b]	R	R	R	R	R	R	R	T	T	T [b]	T [b]
Enterobacteriaceae with inducible ß-lactamases and ESBL	R	R	R	R	R	R	R	R	R	T	T	R	R
Aeromonas with A2 [c] (most *A. sobria*)	R	R	T	T	T	T	T	T	T	R	R	R	R
Aeromonas with A 1 and A 2	R	R	R	R	R	R	T	R	T	R	R	R	R
Ps. aeruginosa *Burkholderia* spp.	R	R	R	R	R	T	T	R	T	T	T	T	T
S. maltophilia	R	R	R	R	R	T	R	R	R	R	R	T	R
A. baumannii	R	T [d]	R	R	R	T	T	R	R	T	T	T	T

AM+CL, Amoxycillin+Clavulanate; AMP, Ampicillin; AZTRM, Aztreonam; CEFUR, Cefuroxime; CETRX, Ceftriaxone; CEZDI, Ceftazidime; CFEPM, Cefepime; CFOXT, Cefoxitin; CFTAX, Cefotaxime, IMIPM, Imipenem; MEROP, Meropenem; PI+TZ, Piperacillin+Tazobactam; TI+CL, Ticarcillin+Clavulanate.

A1 Inducible cephalosporinase, the enzyme is usually found in *A. hydrophila* and *A. caviae*. These species are considered resistant to cephalosporins and cephamycins.
A2 Penicillinase/carbapenemase that hydrolyses imipenem and meropenem. The expression may be heterogeneous.

a) R, the microorganism is resistant and may possess a resistance mechanism not always detected by the diffusion method.
T, these antimicrobials may be used for testing.
b) Test isolates from urine only. Isolates from other sites are considered resistant.
c) Test also for Cefazolin. *A. caviae* does not posses a carbapenemase (A2) and can be tested against imipenem and meropenem.
d) Use Ampicillin+Sulbactam.
e) *K. oxytoca* producing a K-1 enzyme are susceptible to ceftazidime. They may show synergism between other third gen. cephalosporins and amoxicillin+clavulanate and may be mistaken as ESBL producers.

Adopted from CDS 2005 with modifications.

References:

1) Sanders C.C. and Sanders W.E.: Beta-lactam resistance in gram negative bacteria: global trends and clinical impact. Clin. Infect. Dis., **15**, 824-39, 1992.
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16.4 Plasmid-mediated AmpC Beta-lactamases

Plasmid-mediated beta-lactamases represent a new threat, since they confer resistance to aminopenicillins, carboxypenicillins, ureidopenicillins, although they are generally susceptible *in vitro* to mecillinam and/or temocillin.

The enzymes provide resistance to third generation cephalosporins and cefoxitin. The enzymes are also active against aztreonam although for some strains the aztreonam MICs are in the susceptible range. Susceptibility to cefepime is little affected (inoculum effect) and the carbapenems are not affected. The enzymes are not affected by beta-lactamase-inhibitors, except for CMY-8 and CMY-9 that are inactivated by tazobactam.
Their expression is generally constitutive, nevertheless inducible plasmid AmpC (ACT-1, DHA-1, DHA-2, CMY-13) have been reported (6).

Plasmid-mediated AmpC beta-lactamases have been found most frequently in species naturally negative for AmpC, such as *K. pneumoniae*, *E. coli*, *K. oxytoca*, Salmonella and *P. mirabilis*. Recently they were also found in *Enterobacter* spp. (2).

The strains with plasmid-mediated AmpC show resistance to cefoxitin (MIC > 16 µg/ml) and ceftazidime (MIC > 16 µg/ml) corresponding to zones of inhibition < 20 mm (McF. 0.5).

Strains with plasmid-mediated AmpC do not show antagonism between cefoxitin and 3rd generation cephalosporins (are not inducible), while inducible plasmid-mediated AmpC (ACT-1, DHA-1, DHA-2, CMY-13) show antagonism between cefoxitin (or imipenem) and third generation cephalosporins.

Isolated that coproduce an ESBL and a plasmid mediated AmpC beta-lactamase may yield a positive confirmatory test for ESBL using cefepime and cefepime+clavulanate (synergism).

Characteristics of AmpC beta-lactamases:

	Chromosonally mediated AmpC (partially derepressed AmpC mutants)	**Plasmid-** mediated AmpC (derepressed AmpC mutants)	**Inducible plasmid-** mediated AmpC ACT-1, DHA-1, DHA-2, CFE-1, CMY-13	**AmpC** overexpression *P. aeruginosa*
Cefepime+Clavulanate and/or Ceftazidime+Clavula-nate	No synergism	No synergism (except MOX-1, MOX-2)	No synergism	No synergism
Cefoxitin, Imipenem or Amoxycillin+Clavula-nate (7)	Cefoxitin R (zone < 20 mm) **Antagonism** with 3rd gen. cepha.	Cefoxitin R (zone < 20 mm) **No** antagonism with 3rd gen cepha.	Cefoxitin R (zone < 20 mm) **Antagonism** with 3rd gen. cepha.	**No** antagonism Imipenem / 3rd gen cepha.
Ceftazidime Cefepime	S → R S	R (zone < 20 mm) S	S → R S	Ceftazidime R
Cloxacillin 500 µg	**Synergism** Cloxacillin-cefoxitin	**Synergism** Cloxacillin+cefoxitin Cloxacillin+ceftazidime	**Synergism** Cloxacillin+cefoxitin Cloxacillin+ceftazidime	**Synergism** Cloxacillin-ceftazidime
Boronic acid	Boronic acid-ceftazidime	Boronic acid–ceftazidime	Boronic acid–ceftazidime	Boronic acid-ceftazidime

Enterobacter spp., *C. freundii, M. morganii, Hafnia alvei, Providencia* spp., Proteus indole positive *and Serratia marcescens*, all produce an inducible chromosomal AmpC beta lactamase, which is not inhibited by clavulanate. There may be seen an antagonism between amoxycillin and clavulanate (smaller zone with the combination that with amoxycillin alone) due to the presence of the inducible beta-lactamase. All these strains should be reported as resistant to ampicillin/amoxycillin and to amoxycillin+clavulanate (except P. vulgaris).

Using an Amoxycillin+Clavulanate disc (Neo-Sensitabs) better performance is obtained due to the dual action of clavulanic acid: 1) induces expression of inducible plasmid mediated AmpC beta-lactamases (antagonism with 3rd gen. cephalosporins) and 2) permits the detection of an ESBL by enlarging inhibition zones of 3rd gen. cephalosporins (synergism) (7). In the presence of an ESBL + an inducible plasmid-mediated AmpC, both antagonism and synergism can be detected in the same plate (7).

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16.4.1 Differentiation of AmpC beta-lactamases in *E. coli*

Mirelis et al (9) found a simple phenotypic method for the differentiation between plasmid-mediated and chromosomal AmpC-ß-lactamases in *E. coli* using Cloxacillin 500 µg Neo-Sensitabs and by visual examination of the antibiogram plates. The presence of scattered colonies located near the edge of the zone of inhibiton of cefoxitin, cefotaxime, ceftazidime and aztreonam indicated the presence of plasmid-mediated AmpC beta-lactamases.
Cloxacillin 500 µg alone do not distinguish between chromosomal or plasmidic AmpC beta-lactamases.

E. coli
(AMC I/R, Cefotaxime I/R, Ceftazidime I/R Cefoxitin: most I/R)

	Plasmid AmpC	**Inducible plasmid AmpC**	**Chromosomal AmpC hyperprod.**	**Not AmpC**
Cloxacillin 500 µg	Synergy with ceftazidime or cefoxitin	Synergy with ceftazidime or cefoxtin	Synergy with ceftazidime or cefoxitin	No synergy
Boronic acid	Synergy with Ceftazidime+Clav. and/or Cefotaxime+Clav	Synergy with Ceftazidime+Clav. and/or Cefotaxime+Clav	Synergy with Ceftazidime+Clav. and/or Cefotaxime+Clav	-
Cefoxitin Imipenem	No antagonism with 3rd gen. cephalosporins	Antagonism with 3rd gen. cephalosporins	Antagonism or not depending on the degree of derepression	-
Antibiogram	**Scattered colonies** (resistant mutants) near the edge of the zone of cefoxitin, cefotaxime, ceftazidime and aztreonam	**Scattered colonies**	**Well defined edge of zone**	-

The same procedure will be appropriate for *K. pneumoniae* and *P. mirabilis* strains.

Plasmid-mediated AmpC beta-lactamases
MIC µg/ml

Beta-lactamases	**Cefoxitin**	**Ceftazidime**	**Aztreonam**	**Cefepime**	**Imipenem**	**Meropenem**	**Microorganisms**
AAC-1	4-8	≥32	1	0.25	0.125-0.8	0.03	*E. coli, K. pneumoniae, P. mirabilis,* Salmonella, *C. freundii*
ACT-1	> 256	4 - > 128	4 → > 128	≤ 0.06-8	1	·	*E. coli, K. pneumoniae, E. cloacae* (inducible)
BIL-1 (CMY-2)	R	> 16	4-16	1	0.5	0.06	*E. coli*
CMY-1	256	4-128	32	0.25-4	≤ 0.5	0.06	*K. pneumoniae*
CMY-2	32-256	32-128	16-64	0.5-4	≤ 0.5	0.06	*E. coli*, Salmonella, *K. pneumoniae*
CMY-3	128	64	32-256	1	0.25	0.03	*P. mirabilis*
CMY-4	8 - > 256	8-256	0.5-32	0.06-4	0.25	0.125	*E. coli*, Salmonella, *P. mirabilis*
CMY-5	R	256	64	-	0.5-1	-	*K. oxytoca*
CMY-6	256	256	64	0.5	0.25	0.06	*E. coli*
CMY-7	R	> 32	·	I	0.25	< 2	*E. coli*, Salmonella
CMY-8	> 256	32-64	·	·	0.25-0.5	-	*K. pneumoniae*
CMY-9	> 128	128	8	0.25	0.5	0.06	*E. coli*
CMY-10	> 128	8-64	4-128	0.12-0.5	0.25-0.5	≤ 0.125	*E. coli, E. aerogenes, K. pneumoniae*
CMY-11	> 256	256	128	·	·	·	*E. coli*
CMY-12	256	128	8-32	16	0.25-4	0.5	*P. mirabilis*
CMY-13	512	256	64	1	0.25	≤ 0.03	*E. coli* (inducible)
CMY-14	128	128	16-32	0.5-32	0.25-2	0.06	*P. mirabilis*
CMY-15	512	128	8-32	0.25-8	0.25-16	4	*P. mirabilis*
CMY-16	≥ 32	≥ 32	1	2	2	0.05	*P. mirabilis* / Synergy TAZO-FEP
CMY-19	≥ 128	> 128	16	4	0.25	≤ 0.06	*K. pneumoniae* (8)
CMY-21	> 64	64	32	0.5	0.25	0.06	*E. coli*

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Beta-lactamases	Cefoxitin	Ceftazidime	Aztreonam	Cefepime	Imipenem	Meropenem	Microorganisms
CFE-1	R	64	8	0.25	0.25	·	*E. coli* (inducible)
DHA-1	128-512	8-64	1-16	≤ 0.125-2	≤ 0.125 - 0.5	·	*E. coli, K. pneumoniae*, Salmonella, *P. mirabilis* (inducible)
DHA-2	16	8	2	0.03	0.25	·	*K. pneumoniae* (inducible)
FOX-1	128	8	1	1	0.25	≤ 0.03	*E. coli, K. pneumoniae*
FOX-2	256	32	2	0.13	0.5	0.03	*E. coli*
FOX-3	64	16	1	≤ 0.06	0.12	-	*E. coli, K. oxytoca*
FOX-4	> 512	> 128	64	2	0.5	0.12	*E. coli*
FOX-5	512	128	8-16	0.5	0.5	-	*K. pneumoniae, E. coli*
FOX-7	-	-	-	-	-	-	*E. coli, K. pneumoniae, E. cloacae*
LAT-1	64-256	> 128	64	1	0.25-2	0.06	*K. pneumoniae*
LAT-2 (CMY-2)	256	> 256	64-256	-	-	-	*E. coli, K. pneumoniae, E. aerogenes*
LAT-3 (CMY-6)	256	128	64	0.5	0.25	0.06	*E. coli*
LAT-4 (CMY-1)	64-256	8-256	8-128	0.125-1	0.25	0.125	*E. coli*
MIR-1	≥ 256	128	128	1	1	0.125	*E. cloacae, K. pneumoniae*
MOX-1	R	16 → R	16	-	0.5	-	*K. pneumoniae,*
MOX-2	≥ 128	4-256	·	0.25-4	≤ 0.125	-	*K. pneumoniae*

Gupta et al. (3) describes isolation of multiresistant Salmonella, with plasmid-mediated AmpC beta-lactamase, from cattle and humans in the USA.
Three cases of invasive infections caused by *Salmonella enterica* serotype *cholerasuis* found in Taiwan (5). The strains were resistant to ciprofloxacin (mutations gyrA and ParC) and to ceftriaxone (presence of plasmid-mediated CMY-2 beta-lactamase).
Recent studies (4) show that the outcome of cephalosporin treatment in serious infections due to AmpC beta-lactamase producing *K. pneumonia* isolates was poor. A standard test for detection of plasmid-mediated AmpC beta-lactamases is needed.
Emergence of cefepime-hydrolyzing CMY-19 in Japan (8).

Detection of Plasmid-mediated-AmpC beta-lactamases

Strains suspicious of possessing plasmid-mediated AmpC beta-lactamases are cefoxitin resistant (except AAC-1) and have reduced susceptibility to ceftazidime, while currently they are susceptible to cefepime and the carbapenems.

Procedure
Apply Cefotaxime+Clavulanate and Ceftazidime+Clavulanate Neo-Sensitabs. At a distance of 10 mm (edge to edge) of each apply Boronic acid Diatabs.
Apply Ceftazidime and Cefoxitin Neo-Sensitabs. At a distance of 5 to 10 mm (edge to edge) apply Cloxacillin 500 µg Diatabs.

Interpretation
A keyhole or ghost zone (synergism) between Boronoic acid and any of Cefotaxime+Clavulanate or Ceftazidime+ Clavulanate indicates the presence of a AmpC beta-lactamase.
A keyhole or ghost zone (synergism) between Cloxacillin 500 µg and any of Ceftazidime or Cefoxitin indicates the presence of an AmpC beta-lactamase.
Inducible AmpC beta-lactamases (both chromosomal and plasmidic (DHA-1, DHA-2)) will show antagonism (distorted zone) between Cefoxitin and Ceftazidime.
Strains of *Klebsiella* spp., *Salmonella* spp. and *P. mirabilis* that show synergism using Boronic acid and/or Cloxacillin 500 µg possess presumptively plasmid mediated AmpC beta-lactamases. (10).
Strains of *E. coli* showing synergism with Boronic acid and/or Cloxacillin 500 µg may possess plasmidic or chromosomal AmpC-bata-lactamases.
In order to differentiate plasmidic from chromosomal AmpC beta-lactamases in *E. coli* (9), look at the zones around Cefotaxime, Ceftazidime and Aztreonam Neo-Sensitabs. The presence of scattered colonies (resistant mutants) at the edge of the zones indicates plasmid-mediated AmpC, while the presence of well-defined zone-edges indicates chromosomal AmpC beta-lactamase.

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16.5 Inhibitor Resistant TEM Beta-lactamases (IRT)

Strains with this phenotype give patterns of antibiotic resistance similar to TEM 1 or 2 or SHV 1 beta-lactamases, but they are resistant to amoxicillin + clavulanate. IRT are found mainly in *E. coli* and *Klebsiella pneumoniae*.
Are R (resistant) to amoxicillin + clavulanate. Zone diameter for Amoxicillin+Clavulanate Neo-Sensitabs < 19 mm.
Are S (generally susceptible) to cephalosporins: cephalothin, cefoxitin, cefotaxime.

References:
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16.6 Carbapenemases

Carbapenemases are beta-lactamases that significantly hydrolyze at least imipenem and/or meropenem.
Carbapenemases involved in acquired resistance are of Ambler classes A, B and D. They may be plasmid or chromosomally encoded.
Because several of these carbapenemases confer only reduced susceptibility to carbapenems in Enterobacteriaceae, they may remain underestimated, because they are not detected in the laboratory.
Acquired carbapenemases are increasingly reported worldwide and consequently it is important to be able to detect them in the laboratory.

Carbapenemases classification (1)

Ambler classification	Enzymes	MICs µg / ml				Inhibited by		Inducible
		3rd gen cepha	AZTRM	IMIPM	MEROP	CLAV	EDTA	
A	NmcA	S	4	≥ 16	2-8	± wk	no	yes
	Sme-1 to Sme-3	S	4-64	≥ 16	0.25-8	± wk	no	yes
	IMI-1 to IMI-2	S	S	≥ 64	4-32	+	no	yes
	KPC-1 to KPC-4	≥ 32	≥ 64	4→16	4→16	+ or wk	no	no
	GES-2 to GES-5	≥ 32	16→R	0.25→16	0.5-16	+ or 0	no	no
B	IMP 1-16	≥ 32	S→R	0.5-128	0.25→R	no	yes	no
Metallo-beta-	VIM 1-12	≥ 64	S→R	1→R	0.5→R	no	yes	no
lactamases	SPM-1	≥ 256	4	R	R	no	yes	no
	GIM-1	16-32	8-16	> 8	> 8	no	yes	no
	SIM-1	≥ 256	128	8-16	16	no	yes	no
D	OXA 23-27	> 256	> 256	4-64	4-128	± wk	no	·
Oxacillinases	OXA 40-48	S→R	S→R	2-64	0.25-64	wk	no	no
	OXA 54-55	S	S	4	0.25	wk	no	·
	OXA-60	S	R	0.5	2	no	no	yes
	OXA-58	4-128	≥ 32	3-32	2→64	no	no	no

wk = weak

References:
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16.6.1 Detection of acquired carbapenemases Ambler classes A and D

Class A carbapenemases are penicillinases with greater activity against imipenem than meropenem and they also give resistance to penicillins, cephalosporins and aztreonam.
Clavulanate is an inhibitor of class A carbapenemases and therefore synergy with imipenem is useful to detect these enzymes (1,2,3,4,5).
The KPC family of enzymes confer greater resistance to third gen cephalosporins than to carbapenems (3,5).
Carbapenemase IMI-2 is the first inducible and plasmid-encoded carbapenemase.
Class D carbapenemases correspond to the enzymes classified as OXA-types (oxacillinase activity). They hydrolyze imipenem and meropenem weakly and do not hydrolyze third gen cephalosporins and aztreonam (although MICs against the later drugs are often increased due to the presence of other beta-lactamases).

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Clavulanate is a progressive inhibitor of most OXA carbapenemases, but not all. The synergy test (clavulanate and imipenem) may have value for the detection of these enzymes.

KPC possessing *Enterobacter* spp. and *K. pneumoniae* were reported as falsely susceptible to carbapenems using automated systems (Vitek). MIC microdilution using standard inocula of 10^4 or 10^5 CFU/ml did not detect carbapenem resistance, while diffusion methods (E-test) using inocula of 10^8 CFU/ml detected resistance (5,7,12).
K. pneumoniae intermediate or resistant to ertapenem or meropenem should be considered resistant to all carbapenems (7).

Clinicians should be aware of the potential for clinical failure (Class D, OXA-55 carbapenemase) when imipenem is used for treatment of serious infections caused by *S. algae* (9).

Acquired carbapenemases Ambler class A and D

Ambler class.	Enzymes	MICs µg / ml PIPER	3rd gen cepha	AZTRM	IMIPM	MEROP	IMIPENEM + CLAV (synergy)	Inducible	Organisms	Genetic Location
A	NmcA	S	S (0.25-2)	4	≥ 16	2-8	± wk	+	*E. cloacae*	Chromosomal
	Sme-1 to Sme-3	S	0.25-0.5	4-64	≥ 26	0.25-8	± wk	+	*S. marcescens*	Chromosomal
	IMI-1	> 256	1-2	8	> 64	4	+	+	*E. cloacae*	Chromosomal
	IMI-2	16→128	0.1-2	4-8	64	4-32	+	+	*E. asbariae*	Plasmid
	KPC-1	> 128	≥ 32	> 64	16	16	+	no	*K. pneumoniae*	Plasmid
	KPC-2	≥ 64	≥ 8	> 16	8→16	≥ 16	+	no	*K. pneumoniae* /*oxytoca* *Salmonella* *Enterobacter*	Plasmid
(11)	**KPC-2**	·	≥ 256	> 256	256	256	(+)	no	*P. aeruginosa*	Plasmid/Chrom.
	KPC-3	256	256	> 256	> 4	> 4	(+)	no	*K. pneumoniae* *Enterobacter* *E. coli*	Plasmid
	KPC-4				> 16	> 16	(+)		*Enterobacter*	
	GES-2	128	≥ 32	16	4→16	4-16	+	no	*E. cloacae* *P. aeruginosa*	Plasmid, integron
	GES-3	128	64→256	64	0.25	0.5	+	no	*K. pneumoniae*	Plasmid, CEFOX R
	GES-4	128	R	R	8	8	(+)	no	*K. pneumoniae*	CEFOX R
	GES-5	R	R	·	8	8	+	·	*P. aeruginosa*	Integron
D	**OXA-23** to **OXA-27**	> 256	> 256	> 256	4-64	4→128	± wk	·	*A. baumanni*	Chromosomal ± integron
	OXA-40	R	4→128	4→128	> 32	≥ 32	wk	no	*Ac.haemolyticus* *K. pneumoniae* *P. aeruginosa*	Plasmid
	OXA-48	8→R	S→R	S→R	2→64	0.25→64	wk	no	*K. pneumoniae*	Plasmid
	OXA-54	32	S	S	1	0.12	wk	·	*Sh. putrefaciens*	Not integron
	OXA-55	S	S	S	1-4	0.25	no wk	·	*Sh. algae* (9)	Chromosomal
	OXA-58	256	4-128	≥ 32	2-32	2→64	no	no	*A. baumanii*	Plasmid
	OXA-60	S	S	R	0.5	2	no	+	*R. pickettii*	Chromosomal
	OXA-62	S→R	S→R	S→R	2→64	64 →128	no	no	*Pandorea* (10) *pnomenusa*	Chromosomal
	OXA-23, **27**, 49 (subgroup 1)				8→32	8→>32			*Ac. baumannii*	Plasmid (only 23)
D (8)	**OXA-24**, **25, 26, 40** (subgroup 2)								*Ac. baumannii*	Chromosomal
	OXA-51 + OXA-64-66, 68-71, 78-82. OXA-51-like (subgroup 3)				≥ 1	≥ 1			*Ac. baumannii*	Chromosomal
	OXA-58 (subgroup 4)								*Ac. baumannii*	Plasmid (only 58)

Bold = involved in outbreaks

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Procedure for detection of class A and D carbapenemases (14).
Strains with reduced susceptibility to imipenem/meropenem (MIC > 1 ug/ml), I/R to ertapenem (disk) and highly resistant to ceftazidime (KPC and GES enzymes) should be suspected of possessing carbapenemases (particularly KPC). It is important to recognize small resistant colonies growing inside the ertapenem disk zone (13, 24).
Besides Class D enzymes, such as oxacillinases (OXA-25, OXA-34, OXA-58, OXA-64/66) are isolated mainly from *Acinetobacter baumannii* outbreaks. Strains producing Class A enzymes are isolated more frequently (particularly KPC enzymes).

1) Synergy test between Imipenem and Amox+Clav Neo-Sensitabs (distance 10 mm from edge to edge). The addition of 5 % NaCl to the medium provide larger inhibition zones, making the test more sensitive,
2) Synergy test between Imipenem and Imipenem + EDTA Neo-Sensitabs (>6 mm larger zone indicates synergy) .
3) Induction test between Cefoxitin/Imipenem Neo-Sensitabs and Ceftazidime+Dipicolinic acid and Ceftazidime (distance 15 mm from edge to edge).
4) Carbapenem inactivation assay (carbapenemase test) page 140.

Results
Carbapenemases type A will show in most cases a synergism between Imipenem and Clavulanate (KPC/GES). Besides no synergism between Imipenem and EDTA and/or Ceftazidime+Dipicolinic acid (metallo-ß-lactamase negative). Carbapenemase test is positive.
Inducible carbapenemases (Sme-1 to Sme-3, NmcA, IMI-1 and IMI-2, and OXA-60) are induced by cefoxitin and imipenem and will show antagonism between Cefoxitin/Imipenem and Ceftazidime.

Carbapenem inactivation assay. Carbapenemase test (2)
In order to determine whether resistance to carbapenems is caused by a carbapenemase, a tablet (disk) diffusion bioassay may be performed.
A M-H agar plate is inoculated with the susceptible strain *E. coli* ATCC 25922 as for disk diffusion (Mc Farland 0.5 inoculum). Then Imipenem and/or Meropenem Neo-Sensitabs are applied onto the plate.
A suspension of the microorganism to be tested for the presence of carbapenemase is adjusted to McF 0.5 Standard and a loop is used to make a 15-20 mm streak on each side of the Imipenem and/or Meropenem Neo-Sensitabs (starting at the tablet edge). Thereafter incubation for 18-24 hours at 35 – 37 °C.
Alterations in shape of the zones of inhibition around the test organism is considered indication of carbapenemase activity.

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16.6.2 Detection of acquired Metallo-beta-lactamases (MBL)

The worldwide spread of acquired metallo-beta-lactamases (MBL) in gram-negative aerobes is of great concern. MBL production in clinical isolates of key gram-negatives: *P. aeruginosa, E. cloacae, S. marcescens* and *K. pneumoniae* should be carefully monitored (5).

MLBs are classified into 5 major types: IMP, VIM, SPM, and GIM and SIM type enzymes. In Enterobacteriaceae only IMP and VIM enzymes have been found as yet.

MBLs hydrolyze most beta-lactams (carbapenems and large expectrum cephalosporins), except aztreonam. This phenotype of multiple beta-lactam resistance and aztreonam susceptibility may be helpful for identification of these strains in the laboratory. If the strain is resistant to aztreonam it may be due to additional resistance mechanisms (efflux, other beta-lactamases, ESBL etc.). Their expression is not inducible.

The MBL enzymes are resistant to beta-lactamase inhibitors and susceptible to chelating agents like EDTA, 2-mercaptopropionic acid (2-MPA) and Dipicolinic acid (DPA).

Early detection of MBL-producing microorganisms is essential to prevent dissemination of these organisms. The enclosed tables, including strains of Enterobacteriaceae and Non-fermenters producing MBLs, show that MPL-producers (particularly in Enterobacteriaceae) may show low MIC values against carbapenems making it difficult for the laboratory to detect MBL-positive isolates.

Suspicious isolates (resistant to ceftazidime showing no synergy between clavulanate and third gen. cephalosporins and possibly showing reduced susceptibility to carbapenems) should be tested for carbapenemase activity using Imipenem, Meropenem and EDTA and Dipicolinic acid tests.

The first metallo-beta-lactamase producing strain of *E. coli* (in Spain) has been detected in Barcelona, using Imipenem+EDTA Neo-Sensitabs and E-test (3,8). The first metallo-beta-lactamase producing strain of *K. pneumoniae* was found in France (4).

MBL- producing gram-negatives have now emerged in Australia (15).The resistance gene bla-IMP4 appears highly mobile, this outbreak involved 5 different gram-negative genera. Diagnostic laboratories in Australia and other countries must be now in high alert, because early detection may limit the wide dispersal of MBL-genes.

Acquired Metallo-beta-lactamases
NON-FERMENTERS

MBL	3rd gen. Cepha MIC	AZTRM MIC µg/ml	IMIPM MIC µg/ml	MEROP MIC µg/ml	Microorganisms	Genetic location
IMP 1-11	≥ 128	≤ 8/16	≥ 8	≥ 8	*Pseudomonas* spp. *Alcaligenes* spp. *Acinetobacter baumannii*	Chromosomal plasmid integron
IMP 12	≥ 128	32	32	128	*Pseudomonas putida*	
IMP 13-16	≥ 256	4-128	≥ 64	≥ 64	*Pseudomonas aeruginosa*	Integron
VIM 1-3	R	S → R	2-128	1-128	*Achromobacter xylosoxidans* *Pseudomonas aeruginosa* *Pseudomonas putida* (VIM 2 and 4) *Acinetobacter baumannii*	Chromosomal plasmid integron
VIM 4-11	> 256	S → R	32-256	32-256	*Pseudomonas aeruginosa*	
SPM-1	≥ 256	4	R	R	*Pseudomonas aeruginosa*	Plasmid (**not** integron)
GIM-1	16 → 32	8-16	> 8	> 8	*Pseudomonas aeruginosa*	Integron
SIM-1	≥ 256	128	8-16	16	*Acinetobacter baumannii*	Integron
IND-5	1-32	32	32	16	*Chryseobact indologenes*	Chromosomal (23)

MBL are not inhibited by clavulanate, but are inhibited by EDTA or 2-MPA.

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Acquired Metallo-beta-lactamases
ENTEROBACTERIACEAE

MBL	3rd gen. Cepha MIC	AZTRM MIC µg/ml	IMIPM MIC µg/ml	MEROP MIC µg/ml	Microorganisms	Genetic location
IMP-1	≥ 32	< 0.5	2	0.5	*E. coli*	Integron
IMP-1	≥ 32	0.5 → R	4-128	4-128	*S. marcescens, K. pneumoniae, K. oxytoca, E. cloacae / E. aerogenes, Cit. freundii, P. rettgeri, M. morganii,*	Integron
IMP-3	64	0.5	1	·	*Shigella flexneri*	Integron
IMP-4	256	·	3	6	*Citrobacter youngae*	Plasmid
IMP-6	> 128	0.25	2-8	64	*E. coli*	
IMP-6	> 128	128	32	> 128	*Serratia marcescens*	
IMP-8	R	S → R	0.5-8	0.25-4	*Enterobacter cloacae, Klebsiella pneumoniae*	
VIM-1	R	8	8	2	*E. coli, P. mirabilis (integron) C. koseri*	Plasmid
VIM-1	16-128	S → R	1-64	1-32	*Klebsiella pneumoniae, E. cloacae*	Plasmid (integron)
VIM-2	≥ 32	S → R	≥ 1	0.5 → > 2	*Citrobacter freundii / E.cloacae*	Plasmid
VIM-2	≥ 128	32	16-64	8-64	*Serratia marcescens, P. rettgerii*	Integron
VIM-2	8	16	4	0.1 (S)	*Klebsiella oxytoca*	Plasmid (integron)
VIM-4	≥ 32	4 → R	2-4	0.5-1	*K. pneumoniae / E. cloacae*	Plasmid
VIM-12	≥ 128	16	8	4	*K. pneumoniae*	Plasmid (16)
VIM-12	> 32	1	1	0.25	*E. coli*	Plasmid (22)
VIM-2 + GES7					*E. coli*	Integron

MBL are not inhibited by clavulanate, but are inhibited by EDTA or 2-MPA.

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Procedure for MBL detection

Some resistance profiles may suggest MBL production, for example:
a) *Pseudomonas aeruginosa*, *Pseudomonas*. spp. and *Acinetobacter* spp.
All isolates non-susceptible to carbapenems and resistant to either ticarcillim, ticarcillin+clavulanate or ceftazidime should be tested for MBL production.
b) *Enterobacteriaceae*
For *E. coli*, *Klebsiella* spp., *P. mirabilis*, *Salmonella* spp. and *Shigella* spp.: All carbapenem S-I-R isolates that are resistant to cefoxitin and amoxicillin+clavulanate and are non-susceptible to ceftazidime (inhibition zone < 18 mm) should be tested for MBL production. In all other cases all isolates are non-susceptible to carbepenems (18).

Procedure
Apply an EDTA Diatabs on the plate. At 10 mm distance apply Imipenem Neo-Sensitabs at one side and Meropenem at the other side (10 mm distance). In case the strains shows no zone around Imipenem/Meropenem the distance to EDTA should be reduced to 5 mm.
Apply Ceftazidime Neo-Sensitabs and Dipicolinic acid (DPA) Diatabs at a distance of 5 mm from each other. Apply Imipenem+EDTA and Ceftazidime+DPA separate from the others.

Interpretation
The use of 2 chelating agents (EDTA and DPA) will enhance the detection of metallo-beta-lactamases in the clinical laboratory (25).
A key-hole or ghost zone (synergism) between EDTA and Imipenem and/or Meropenem indicates the presence of a MBL (19).
A key-hole or ghost zone between Ceftazidime and Dipicolinic acid indicates the presence of a MBL.
A difference of ≥ 5 mm larger zone with Imipenem+EDTA compared to Imipenem and EDTA alone indicates the presence of a MBL. False MBL-positive results (9,15) may be obtained if EDTA alone is not used for comparative zone results with Imipenm+EDTA (due to the intrinsic antibacterial activity of EDTA).
A difference of ≥ 5 mm larger zone with Ceftazidime+DPA compared to Ceftazidime alone indicates the presence of a MBL.
Strains showing no zone of inhibition around Ceftazidime Neo-Sensitabs and a zone of inhibition ≥ 13 mm around Ceftazidime+DPA Neo-Sensitabs (synergism) should indicate the presence of a MBL.

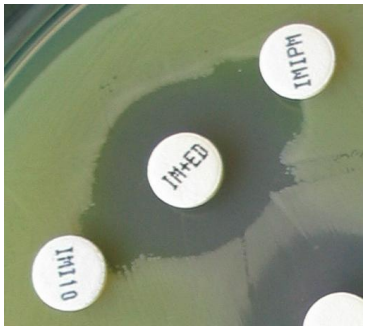

Plate 16.6-a *Pseudomonas aeruginosa* CCUG 51971 producing metallo-beta-lactamases.
Note the large zone around Imipenem+EDTA Neo-Sensitabs (IM+ED) reflecting the production of metallo-beta-lactamases and no zone around Imipenem Neo-Sensitabs (IMIPM); visible at the left and right side of IM+ED there is synergism between both Imipenem Neo-Sensitabs (different potencies) and the EDTA component of the combination of IM+ED.

Plate 16.6-b

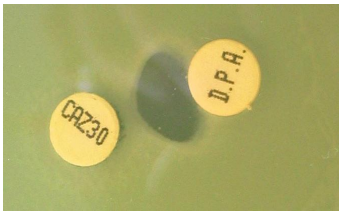

Plate 16.6-c

Demonstration of the presence of metallo-beta-lactamases in *Pseudomonas aeruginosa* CCUG 51971 using discs with dipicolinic acid (D.P.A.); the combination ceftazidime plus D.P.A. or D.P.A. alone.
Plate 16.6-b: Note the large zone around Ceftazidime+D.P.A. (CAZ+D) and no zone around Ceftazidime (CAZ30) reflecting the production of metallo-beta-lactamases.
Plate 16.6-c: Note the synergism between Ceftazidime (CAZ30) and D.P.A. and no zone around Ceftazidime (CAZ30) or D.P.A. reflecting the production of metallo-beta-lactamases.

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16.7 Detection of multiple beta-lactamases in one strain

Diagnostic problems posed by coexistance of different classes of beta-lactamases in a single bacterial isolate could be solved by the combined use of various phenotypic detection methods. See below example with multiresistant *K. pneumoniae* from Taiwan and USA.

	Neo-Sensitabs				
***K. pneumoniae* producing:**	**Cefoxitin**	**Cefepime**	**Ceftazidime+ Clavulanate or Cefepime+ Clavulanate synergy**	**D.P.A. + Ceftazidime or Imipenem+ EDTA synergy**	**Boronic 250 µg Cefoxitin+Clav/ Cefepime+ Clav. or Cloxacillin 500 µg Cefoxitin/Ceftazidime synergy**
AmpC	R	S	negative	negative	POSITIVE
ESBL	S (V)	I / R	POSITIVE	negative	negative
AmpC + ESBL	R	I / R	POSITIVE	negative	POSITIVE
AmpC + metallo-ß-lactamases	R	I / R	negative	POSITIVE	POSITIVE
AmpC + ESBL + metallo-ß-lactamases	R	I / R	POSITIVE	POSITIVE	POSITIVE

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16.8 Detection of ß-lactam Resistance Phenotypes

16.8.1 Detection of ß-lactam Resistance Phenotypes in Enterobacteriaceae

	AMP	AMC	CLOT	CAZ	FEP	FOX	CAZ+CLAV FEP+CLAV	IMI	IMI+ EDTA CAZ+DPA	CLOXA 500 Boronic	Comments
1) *E. coli* / *P. mirabilis* / *Salmonella* spp. / *Shigella* spp. / *Klebsiella* spp., /*C. diversus*											
Penicillinase low (*E. coli*)	I/R	S/I	S	S	S	S		S			
Penicillinase high	R	R	R	S	S	S		S			
Cephalosporinase low	S/I	S/I	I/R	S	S	S		S			
AmpC **high / plasmid**	R	R	R	I/R	S	I/R		S		synergy CAZ+ CLAV	FOX antagonism with CAZ or FEP indicates inducible
AmpC + ESBL	R	R	R	R	S/R	R	Synergy	S		synergy CAZ+ CLAV	
IRT	R	R	S/I	S	> CAZ (zone)			S			
Oxacillinase	R	R	S/I	S	≤ CAZ (zone)			S			
Penicillinase + Cephalosporinase	R	R	R	S/I	S	I/R		S			
Chromosomal K-1 high (*Klebsiella oxytoca*)	R	I/R	R	S	S/I	S	false synergy FEP+CLAV	S			
ESBL	R	V/R	I/R	V/R	V/R	S/R	synergy	S			
Metallo-ß-lactamase	R	R	R	I/R	I/R	R	-	S/R	Synergy		AZT S^R
Carbapenemase class A	R	S/R	R	I/R	I/R	R		I/R (syn CLAV)			
2) *Enterobacter* spp. / *C. freundii*											
Penicillinase	R	R	R	S	S	R		S			
AmpC derepressed	R	R	R	R	S	R		S		syn FOX syn CAZ + CLAV	
AmpC + ESBL	R	R	R	R	S/R	R	Synergy	S		syn FOX syn CAZ + CLAV	
ESBL, Metallo-ß-lactamase and Carbapenemases	As for *E. coli* etc.										
3) *M. morganii* / *Providencia* spp. / *Serratia* spp.											
Penicillinase	R	R	R	S	S	R		S			
Amp C derepressed	R	R	R	R	S	I/R		S		syn FOX syn CAZ + CLAV	
ESBL, Metallo-ß-lactamase and Carbapenemases	As for *E. coli* etc.										
4) *P. vulgaris* / *P. penneri*											
Penicillinase	R	S	R	S	S	S		S			
Chromosomal ß-lactamase derepressed	R	S	R	R	S	S		S			

AMP; Ampicillin, AMC; Amoxicillin – clavulanic acid, CLOT; Cephalothin, CAZ; Ceftazidime, FEP; Cefepime, FOX; Cefoxitin, CAZ+CLAV; Ceftazidime + clavulanic acid, FEP+CLAV; Cefepime + clavulanic acid, IMI; Imipenem, IMI+EDTA; Imipenem+EDTA, Cloxa 500; Cloxacillin 500 µg (High), AZT; Aztreonam.

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16.8.2 Detection of ß-lactam Resistance Phenotypes in Non-fermenters

	TIC	TCC	PIP	CAZ	FEP	IMI	MERO	CAZ+CLAV FEP+CLAV	IMI+EDTA CAZ+DPA	Remarks
1) *Pseudomonas aeruginosa*										
Penicillinase (TEM 1-2, PSE 1-4)	R	S/I	I/R	S	S	S	S			
Amp C partial derep.	S/I	any	I	I	S/I	S	S			
Amp C derepressed	I/R	R	I/R	R	I/R	S	S			CAZ < AZT TIC > CTAX
Oxacillinase	I/R	I/R	any	S	S/R (≤CAZ)	S	S			FEP ≤ CAZ TIC > CTAX
OXA-31, OXA 1, 4.	I/R	I/R	any	S	I/R	S	S			
Mex XY-OprM Efflux I/R (1)				S	I/R	S	S			CAZ ≥ FEP
PER 1-2, VEB-1 (ESBL)	R	S	S/I	R	R	S (PER-1) I/R (VEB-1)	S (PER-1) I/R (VEB-1)	Synergy		Phenotype PIP S, CAZ R indicates PER-1 enz.
ESBL	I/R	any	R	I/R	R	S	S	Synergy		
Metallo-ß-lactamase	R	R	R	R	R	I/R	I/R		Synergy	AZT, S^R
Carbapenemase Class A	R	S/I	R	I/R	I/R	I/R	I/R			AZT, S^R Syn. IMI+CLAV
Increased efflux	I/R	I/R	I/R	I/R	I/R	S^R	I			CAZ > AZT
Loss of Opr porin	S	S	S	S	S	I/R	S/I			
2) *Stenotrophomonas maltophilia*										
Beta-lactamase L-1	R	R	R	R	R	R	R	-	Synergy	AZT, S
Beta-lactamase L-2	-	S	I	R	R	S	-	-	-	AZT, R
Beta-lactamase L-1+L-2	R	R	R	R	R	R	R	-	Synergy	AZT, R
3) *Acinetobacter baumannii*										
	TIC	**PIP**	**PTZ**	**CAZ**	**FEP**	**IMI**	**MERO**			
Penicillinase (TEM 1-2)	R	R	S	S	S	S	S			
Oxacillinase (OXA 21, 37)	R	R	S	S	S (≤CAZ)	S	S			FEP ≤ CAZ
Amp C partial derep.	I	I	S/I	I	S/I	S	S			
Amp C derepressed	R	R	I/R	R	I/R	S	S			
PER-1, VEB-1 (ESBL)	R	any	S	R	R	S	S	Synergy		Syn. TIC+CLAV Syn. PIP+TAZO
ESBL	R	R	any	R	R	S	S	Synergy		
Metallo-ß-lactamase	R	R	R	R	R	I/R	I/R		Synergy	AZT, S^R
Carbapenemase Class D (Oxa 23-27, 40, 51, 58)	R	R	I/R	I/R	I/R	S/I/R	S/I/R			AZT, S^R
Loss of porins	S	S	S	S	S	I/R	S/I			

TIC; Ticarcillin, TCC; Ticarcillin – clavulanic acid, PIP; Piperacillin, PTZ; Piperacillin – tazobactam, CAZ; Ceftazidime, FEP; Cefepime, IMI; Imipenem, MERO; Meropenem, CAZ+CLAV; Ceftazidime + clavulanic acid, FEP+CLAV; Cefepime + clavulanic acid, IMI+EDTA; Imipenem + EDTA, AZT; Aztreonam.

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17 Detection of Other Resistance Mechanisms

17.1 Screening of 16S rRNA Methylases (HLR to Aminoglycosides)

Unlike aminoglycoside-modifying enzymes that vary in their substrate profile, the acquired 16S rRNA methylases confer high level resistance (HLR) to almost all clinically important aminoglycosides.
They have been identified in several nosocomial pathogens, including *P, aeruginosa*, *Serratia marcescens*, *E. coli*, *P.* mirabilis, *K. pneumoniae* and *Acinetobacter* spp., *Enterobacter cloacae*, *Citrobacter freundii* (8,9).
These enzymes (RmtA, RmtB, RmtC, ArmA) are capable of conferring very high levels of resistance (MIC > 512 µg/ml) against amikacin, gentamicin, isepamicin, netilmicn and tobramycin, while apramycin, neomycin and streptomycin are not affected. The responsible genes ArmA, RmtA, RmtB and RmtC are located in self-tramsmissible plasmids (7).

Screening method
A high-level amikacin resistance (MIC > 512 µg/ml) corresponding to no-zone of inhibition around Amikacin 40 µg Neo-Sensitabs may be used as a marker for screening the 16S rRNA methylase producing strains.
The diffusion test is performed on MH-agar using a 0.5 McF inoculum and incubation at 35-37 °C overnight.
Strains of Enterobacteriaceae and non-fermenters (*P. aeruginosa* and *Acinetobacter* spp.) showing no-zone of inhibition around Amikacin 40 µg Neo-Sensitabs should be suspected of possessing 16S rRNA methylases.

a) Enterobacteriaceae
16S rRNA methylase **positive** strains will show:

Amikacin 40 µg:	No zone of hibition
Gentamicin 40 µg:	No zone of hibition
Netilmicin 40 µg:	No zone of hibition
Tobramycin 40 µg:	No zone of hibition
Neomycin 120 µg:	Zone of inhibition ≤ 20 mm or no zone
Apramycin 40 µg	Zone of inhibition ≥ 20 mm (6) (S)

b) Non-fermenters
16S rRNA methylase **positive** strains will show:

Amikacin 40 µg:	No zone of hibition
Gentamicin 40 µg:	No zone of hibition
Netilmicin 40 µg:	No zone of hibition
Tobramycin 40 µg:	No zone of hibition
Neomycin 120 µg	No zone or small zone
Streptomycin 100 µg:	Small zone in most cases

Galimand et al (5) found in 12 clinical isolates of Enterobacteriaceae the armA gene associated with ESBL beta-lactamase CTXM-3 (cefotaxime zone < ceftazidime zone) on a conjugative plasmid.

Bogaerts et al (9) investigated the presence of 16 SrRNA methylase mediated high level resistance to aminoglycosides in clinical isolates of Enterobacteriaceae from 2 University Hospitals in Belgium. They screened for HLR to gentamicin, tobramycin and amikacin resistance and deleted by PCR, ArmA genes in 18 *K. pneumoniae*, *E. coli*, *E. aerogenes*, *E. cloacae*, and *C. amaloneticus*, whereas RmtB was detected in a single *E. coli* isolate. These strains were susceptible to Apramycin and Neomycin Neo-Sensitabs (except 2 strains). All 16 SrRNA methylase positive strains produced ESBL's predominantly type CTX-M3.

The emergence of 16S rRNA methylases in Enterobactericaceae and non-fermenters (*P. aeruginosa*, *Acinetobacter* spp.) in strains that already are ESBL positive, may result in the spread of multidrug-resistant isolates producing both ESBLs and 16S rRNA methylases becoming an important clinical problem.

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17.2 Screening for Plasmid-mediated Quinolone Resistance (*QnrA, QnrB, QnrS*) in Enterobacteriaceae. Integrons

The plasmid gene responsible for quinolone resistance (*QnrA, QnrB, QnrS*) is carried on class 1 integrons of the In 4 family, an efficient mechanism for rapid horizontal and vertical dissemination og antibiotic resistance determinants among bacteria.
The plasmid mediated mechanisms have led to resistance to almost all clinical important antimicrobials, such as β-lactams, aminoglycosides, macrolides, phenicols, sulphonamides and trimethoprim.

The identification in the US of *qnr* in clinical strains of *K. pneumoniae* isolates besides producing plasmidic β-lactamases and ESBL's (7) and its discovery in strains of *E. coli* from Southeast Asia and Salmonella in Hong Kong indicates the emergence of this new mechanism of quinolones resistance in clinical strains.
It is important to indicate that a significant relation exists between quinolone resistance and resistance to 3. gen. cephalosporins (co-resistance): ESBL and/or plasmid mediated AmpC (6,14).

Poirel et al (10) have shown in vivo selection of fluoroquinolone-resistant E. coli isolates expressing plasmid mediated quinolone resistance and ESBL and physical linkage between ESBL and QnrA-encoding genes in the same integron.

Although *QnrA, QnrB, QnrS* produce low levels of quinolone resistance, it facilitates selection for a high level of quinolone resistance.

QnrB, another plasmid-mediated gene for quinolone resistance has been discovered in plasmids encoding the ESBL: CTA-15 from a K. pneumoniae. These strains show low-level resistance to quinolones and MIC of 16 µg/ml towards nalidixic acid, and show similar multiresistance phenotypes as Qnr A containing strains (11).

Lavigne et al (12) screened for qnr genes 112 clinical isolates of ESBL-producing *E. coli* from French hospitals in 2004. 7.7 % of CTX-M-producing *E. coli* presented a plasmid-mediated resistance to quinolones. All strains harboured a qnrA gene located on a class 1 integron.

Poirel et al (13) listed 186 ESBL positive Enterobacteriaceae. From them 2.2 % and 1.6 % carried a QnrA1 and a QnrS1 determinant respectively. The association of the QnrA gene with class 1 integrons was confirmed.

Hyunjoo Pai et al (14) screened *E. coli* and *K. pneumoniae* producing ESBLs or plasmid mediated AmpC beta-lcatamases for the presence of qnrA and qnrB genes. QnrB was present in 54 of 54 DHA-1 producing *K. pneumoniae* isolates and 10 of 45 SHV-12 producing isolates. It is possible that qnrB contributes to the widespread sistribution of DHA-1 (plasmid mediated AmpC) in areas, where 3rd generation cephalosporins and fluoroquinolones are widely used.

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Screening procedure
Perform antibiogram as usual (standard procedure): MH agar, inoculum McF 0.5, incubation at 35-37 °C for 18-24 hours.
Strains of Enterobacteriaceae should be suspected of plasmid-mediated quinolone resistance when showing unusual multiresistance phenotypes such as:

Neo-Sensitabs

Ampicillin 33 µg	- no zone (HLR)
Sulphonamides	- no zone (HLR)
Trimethroprim	- no zone (HLR)
Trimethroprim+Sulfa	- no zone (HLR)
Streptomycins	- no zone (HLR)
Nalidixan	- no zone or zone < 20 mm
Ceftazidime	- zone < 20 mm
Chloramphenicol	- may show resistance
Gentamicin	- may show resistance
Tetracyclines	- may show resistance

Suspected strains can be tested for the presence of the Q*nr* gen by PCR.
It should be noted that strains showing the above-mentioned resistance phenotypes are most probably integron-carrying. Enterobacteriaceae and barrier precaution should be established to prevent further spread.

In a selected group of ciprofloxacin and ceftazidime-resistant Enterobacteriaceae (mainly *K. pneumonae* and *E. cloacae*), carriage of QnrA gene was 32 % (9). From those 73 % were ESBL-positive.

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17.3 Detection of Resistance Mechanisms (General)

Mechanisms of resistance include production of inactivating enzymes, alteration of drug targets, and altered drug uptake or efflux. Find enclosed in the table below the antibiotics recommended to detect certain resistance mechanisms (1).

Antibiotic (Neo-Sensitabs)	**Phenotype**	**Mechanism of resistance**	**Bacteria**
1) Beta-lactams			
Penicillin + pH indicator (Beta-lactamase - D.T.)	Penicillin resistance	Penicillinase	Staphylococci, Haemophilus, Gonococci
Oxacillin 1 µg	Resistance to all beta-lactams	Additional PBP	Staphylococci
Cefoxitin 60 µg (zone < 24 mm)	Resistance to all beta-lactams	mecA	*Staphylococcus aureus*
Ampicillin 33 µg (zone < 20 mm)	a) Resistance to penicillins and beta-lactams inhibitor comb.	Altered PBPs	Enterococci
Oxacillin 1 µg (zone < 20 mm) (zone < 14 mm) (zone < 12 mm)	Penicillin resistance	PBP alteration	Pneumococci, Streptococci, Gonococci
Ceftizoxime (zone < 26 mm)	Resistance to third generation cephalosporins	PBP alteration	Pneumococci
Ampicillin 2.5 µg (zone < 20 mm)	Resistance to AMP, AMOXY, AM+CL, CCLOR, CEFUR (BLNAR strains)	PBP alteration	Haemophilus
Amoxycillin and Amoxycillin+Clavulanate	b) Penicillin resistance AM+CL synergy	Beta-lactamase (BRO-1, BRO-2)	*Moraxella catarrhalis*
Ampicillin 33 µg and Amoxycillin+Clavulanate	Penicillin resistance and AM+CL susceptibility	Penicillinase	Enterobacteria
Ceftazidime, Ceftriaxone (zone < 24 mm)	Resistance to all cephems and aztreonam	Screening ESBL	*Klebsiella* spp, *E. coli*, Salmonella
Cefpodoxime (zone < 20 mm)	Resistance to all cephems and aztreonam	Screening ESBL	*E. coli*, Klebsiella Salmonella
Cefotaxime, Ceftriaxone Ceftazidime/Cefepime and Amoxycillin+Clavulanate	c) Synergy between CFTAX, CETRX, CEZDI and AM+CL, CP+CL, (double disk synergy)	Extended spectrum beta-lactamase (ESBL)	Enterobacteriaceae d)
Ceftazidime+Clavulanate	CZ+CL zone ≥ 5 mm than CEZDI alone	ESBL	Enterobacteriaceae
Cefepime and Amoxycillin + Clavulanate	Synergy between CFEPM and AM+CL	ESBL	Enterobacter, Serratia, *Citrobacter freundii*
Cefepime+Clavulanate	CP+CL zone ≥ 5 mm than CFEPM alone	ESBL (confirmatory)	Enterobacteriaceae
Cefoxitin + Cephalosporins Imipenem + Cephalosporins	Antagonism, indicates cephalosporin resistance	Inducible cephalosporinase AmpC	Enterobacteriaceae
Cefepime+Clavulanate Cefoxitin/3rd gen. cepha.	No synergy No antagonism Cefoxitin R, Ceftazidime R	Plasmid mediated AmpC	Enterobacteriaceae
Amoxycillin+Clavulanate (Zone < 19 mm) Cefazolin	Amoxycillin+Clavulanate R Cefazolin S	Inhibitor resistant TEM ß-lactamase	*E. coli* Klebsiella

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Antibiotic (Neo-Sensitabs)	Phenotype	Mechanism of resistance	Bacteria
Cefoxitin	Antibiotic resistance	Porin alteration	*E. coli* Klebsiella
Aztreonam, Ceftazidime, Cefepime and Ticarcillin + Clavulanate	Synergy between TC+Cl and AZTRM, CFEPM, CEZDI.	ESBL	*Ps. aeruginosa*
Imipenem+EDTA	Synergy between Imipenem and EDTA	Metallo-ß-lactamase	*Ps. aeruginosa* Acinetobacter *E. coli*
Cloxacillin 500 µg	Synergy between cefoxitin/ ceftazidime and cloxacillin 500 µg	AmpC beta-lactamase	Enterobacteriaceae
Dipicolinic acid	Synergism with Ceftazidime and/or Imipenem	Metallo-ß-lactamase	Enterobacteriaceae Non-fermenters
Boronic acid	Synergism with Cefotaxime+Clav. and/or Ceftazidime+Clav.	AmpC beta-lactamase	Enterobacteriaceae
2) Aminoglycosides			
Kanamycin 100 µg (zone < 25 mm)	Amikacin and Isepamicin resistance	APH(3'), ANT(4')	Staphylococci
Gentamicin 40 µg (zone < 23 mm)	Resistance to aminoglycosides except streptomycin	APH(2'')-AAC(6')	Staphylococci
Kanamycin 500 µg (zone < 14 mm)	HLR to amikacin (no synergy with penicillins)	APH(3'), ANT(4')	Enterococci (HLR)
Gentamicin 250 µg (zone < 14 mm)	HLR to all aminoglycosides	APH(2'')-AAC(6')	Enterococci (HLR)
Streptomycin 500 µg (zone < 14 mm)	Streptomycin resistance		Enterococci (HLR)
Amikacin + Tobramycin	Resistance to aminoglycosides	APH(3')-VI	Acinetobacter
Netilmicin + Tobramycin	Resistance to aminoglycosides	AAC(3)	Pseudomonas
3) Others			
Erythromycin + Clindamycin	Inducible MLS resistance (antagonism)	Ribosomal methylation	Staphylococci, Streptococci,
Nalidixan (zone < 25 mm)	Reduced sensitivity to quinolones	DNA gyrase	Enterobacteriaceae *Vibrio cholerae* Haemophilus
Nalidixan (zone < 28 mm)	Reduced sensitivity to quinolones	DNA gyrase	Gonococci Meningococci
Ciprofloxacin 0.5 µg (zone < 20 mm)	Quinolone resistance	DNA-gyrase	Gonococci, Haemophilus
Vancomycin 5 µg (Teicoplanin 30)	Vancomycin resistance 2+18 hours' prediffusion	Van A, Van B VISA, hVISA	Enterococci, Staphylococci
Metronidazole	Imidazole resistance	Reductase	Anaerobes

a) for non beta-lactamase producing enterococci.

b) Synergy: AM+CL zone > 5 mm larger than AMOXY (resistance to penicillin, amoxycillin and ampicillin, susceptible to amoxycillin+clavulanate).

c) Beta-lactam resistance (except cephamycins and carbapenems).

d) Except *Proteus penneri* and *P. vulgaris*.

Note: The mentioned zone sizes are valid for McFarland 0.5 inoculum.

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17.4 Intrinsic (Natural) Resistance

Antimicrobial resistance can be classified as either intrinsic or acquired. Intrinsic resistance may be related to inherent or natural characteristics in a bacteria and may be used for recognition of a bacterial species, and results of in vitro susceptibility testing is not relevant to report as treatment options. The most relevant drug related natural resistance in a group or species is listed below:

BACTERIA	**NATURAL RESISTANCE**
Enterobacteriaceae	
Enterobacteriaceae in general	Penicillinase stable penicillins, Macrolides, Fucidin, Rifampicin, Glycopeptides
Enterobacteria, group 2 *K. pneumoniae, K oxytoca, C. diversus, Esch. hermannii*	Aminopenicillins, Carboxypenicillins
Enterobacteria, group 3 *E. cloacae, E. aerogenes, C. freundii, S. marcescens, M. morganii, Prov. rettgeri, P stuartii, Hafnia alvei* (except *P. vulgaris* AMC S)	Aminopenicillins, Amoxicillin+Clavulanate, 1st gen Cephalosporins
Citrobacter freundii	Aminopenicillins, Amoxicillin+Clavulanate, 1st gen Cephalosporins, Cefoxitin,
Citrobacter koseri (diversus)	Aminopenicillins, Carboxypenicillins
Enterobacter aerogenes, E cloacae	Aminopenicillins, Amoxicillin+Clavulanate, Cefoxitin, 1st gen Cephalosporins, Nitrofurantoin
Klebsiella pneumoniae, K. oxytoca	Aminopenicillins, Carboxypenicillins
Morganella morganii	Aminopenicillins, Amoxicillin+Clavulanate, 1st and 2nd gen Cephalosporins, Cefoxitin, Polymyxins, Tetracyclines, Nitrofurantoin
Proteus mirabilis	Polymyxins, Tetracyclines, Nitrofurantoin
Proteus vulgaris, P. penneri	Aminopenicillins, Carboxypenicillins Cefuroxime, Polymyxins, Tetracyclines, Nitrofurantoin
Providencia rettgeri	Aminopenicillins, Polymyxins, Tetracyclines, Nitrofurantoin, Amoxycillin+Clavulanate.
Providencia stuartii	Aminopenicillins, Amoxicillin+Clavulanate, Polymyxins, Tetracyclines, Nitrofurantoin, Gentamicin, Tobramycin, Netilmicin
Salmonella spp.	1st and 2nd gen Cephalosporins, Cefuroxime (active in vitro, not active in vivo) Aminoglycosides (in vivo)
Serratia marcescens	Aminopenicillins, Amoxicillin+Clavulanate, 1st and 2nd gen Cephalosporins Polymyxins
Shigella spp.	1st and 2nd gen Cephalosporins, Aminoglycosides (in vivo)
Yersinia enterocolitica	Aminopenicillins, Carboxypenicillins, Amoxicillin+Clavulanate, 1st and 2nd gen Cephalosporins, Cefoxitin
Gram positive cocci in general	Aztreonam, Nalidixic acid, Polymyxins
Staphylococci	
Staphylococcus spp. in generel	Nalidixic acid, Polymyxins
S. saprophyticus	Novobiocin, Fosfomycin
Penicillin resistant staphylococci (Oxa S)	Penicillin, Aminopenicillins, Ureidopenicillins, Carboxypenicillins
Methicillin resistent staphylococci	All beta-lactams
Micrococcus spp.	Nitrofurantoin, Mupirocin

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BACTERIA	NATURAL RESISTANCE
Streptococci/enterococci	
Streptococcus spp.	Polymyxins, Nalidixic acid, Aminoglycosides (low level)
Enterococcus faecalis	Cephalosporins, Clindamycin, Mupirocin, Aminoglycosides (low level – HLR test), Novobiocin, Trim+Sulfa (in vivo)
E. faecium	Cephalosporins, Aminoglycosides (low level – HLR test), Nitrofurantoin, Trim+Sulfa (in vivo)
E. gallinarum/casseliflavus	Vancomycin (MIC 4-16 µg/ml)
***Arcanobacterium* spp**.	Bacitracin, Mupirocin, Optochin
Pediococcus/Leuconostoc Lactobacillus/Erysipelothrix	Glycopeptides
Non fermenters	
Acinetobacter baumanii/calcoaceticus	Aminopenicillins, 1st and 2nd gen Cephalosporins, Chloramphenicol, Trimethoprim, Fosfomycin, Nitrofurantoin
Achromobacter xylosoxidans	Aminopenicillins, 1st and 2nd gen Cephalosporins, Aminoglycosides, Aztreonam
Alc denitrificans	Cefotaxime
Burkholderia cepacia	Aminopenicillins, Ureidopenicillins, Carboxypenicillins, Amoxicillin+Clavulanate, 1st and 2nd gen Cephalosporins, Quinolones, Aminoglycosides, Polymyxins, Nitrofurantoin, Fosfomycin, Chloramphenicol
Chryseobacterium meningosepticum	Aminoglycosides, Carboxypenicillins, 1st, 2nd and 3rd gen. Cephalosporins, Polymyxins, Tetracyclines, Chloramfenicol, Ticarcillin+Clavulanate, Quinolones
Ochrobactrum anthropi	Ureidopenicillins, Carboxypenicillins, Ticarcillin+Clavulanate, 3rd gen. Cephalosporins, Aztreonam
Pseudomonas aeruginosa	Aminopenicillins, Amoxicillin+Clavulanate, 1st and 2nd gen. Cephalosporins, Cefotaxime, Ceftriaxone, Chloramfenicol, Nalidixic acid, Trim+Sulfa, Tetracyclines, Nitrofurantoin
Stenotrophomonas maltophilia	Ureidopenicillins, Carboxypenicillins, 1st and 2nd gen Cephalosporins, Imipenem, Cefotaxime, Aztreonam, Aminoglycosides, Tetracyclines (except Minocycline) Fosfomycin
Listeria	Oxacillin, Cephalosporins, Aztreonam, Polymyxins, Nalidixic acid, Clindamycin, Fosfomycin
Neisseria/Branhamella	
Branhamella catarrhalis	Lincomycin, Clindamycin, Trimethoprim
Gonococci, meningococci	Lincomycin, Clindamycin, Polymyxins, Trimethoprim, Vancomycin
Campylobacter/Helicobacter	
Campylobacter spp.	Vancomycin, Trimethroprim, Polymyxins, Lincomycin, Novobiocin, Aztreonam
Helicobacter pylori	Vancomycin, Polymyxins, Nalidixic acid, Trimetoprim, Sulfonamides
Corynebacterium in general	Fosfomycin, Mupirocin, Polymyxins, Nalidixic acid
C. jeikeium/urealyticum	All Penicillins, 1st, 2nd and 3rd gen. Cephalosporins, Amoxicillin+Clavulanate., Imipenem, Meropenem, Aminoglycosides, Chloramphenicol, Nalidixic acid, Trim+Sulfa, Polymyxins, Fosfomycin, Mupirocin, Macrolides
Anaerobes in generel	Aminoglycosides, Aztreonam (exept Fusobacteria), Trimethoprim, Nalidixic acid
Bacteroides fragilis group	Aminoglycosides, Vancomycin, Aminopenicillins, 1st, and 2nd gen. Cephalosporins, Polymyxins, Glycopeptides, Fosfomycin, Aztreonam, Oxgall
Clostridium spp.	Kanamycin, Trimethrprim, Aztreonam, Polymyxins, Fosfomycin
Fusobacteria spp.	Nalidixic acid, Vancomycin, Macrolides (low level)
Porphyromonas spp	Polymyxins, Fosfomycin, Aminoglycosides
Prevotella spp.	Glycopeptides, Fosfomycin, Aminoglycosides

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BACTERIA	NATURAL RESISTANCE
Actinomyces/Propionibacterium	1st, and 2nd gen. Cephalosporins, Polymyxins, Metronidazole
Mobiluncus spp.	Metronidazole
Peptostreptococcus/Eubacterium	Polymyxins, Fosfomycin
Veillonella spp.	Macrolides (low level), Glycopeptides

References:

1) Livermore DM et al. Interpretative reading: recognizing the unusual and inferring resistance mechanisms from resistance phenotypes. J Antimicrob Chemother. 2001; 48 Suppl 1:87-102.
2) Comminique January 2005: Societé Francaise de Microbiologie (CA-SFM)

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18 Sources of Error in the Diffusion Test

The following common sources of error should be considered, when a zone diameter is outside the indicated control limits with ATCC Control Strains:

- Variability in the agar test medium (lot variation).
- pH value of medium too high or too low.

  If the pH value is too low: Zones of inhibition will be smaller with aminoglycosides, macrolides and quinolones.
  If the pH value is too high: Zones of inhibition will be too small for penicillins and tetracyclines.
- Ca^{++} and Mg^{++} content of media.

  - content too low: Zone too small with daptomycin
    Zone too large with *P. aeruginosa* and aminoglycosides
  - content too high: Zone too small with *P. aeruginosa* and aminoglycosides
    Zone too small with Tetracyclines
- Inoculum used, too heavy or too light.
- Inoculum for the test prepared from a plate incubated for too long time (more than 24 hours old).
- Incorrect incubation temperature and/or atmosphere used.
- Contamination of the control strain.
- Deteriorated McFarland standard or failure in the vortex procedure of the $BaS0_4$ turbidity standard.
- Loss of disk potency, which is unlikely with Neo-Sensitabs.
- Error in reading the inhibition zones.
- Error in transcribing the data.

Some errors can be readily resolved by examining the test plates. If no obvious deviations are found, daily control tests should be performed for at least 5 consecutive test days or until the problem is resolved (1).

References:

1) NCCLS. Performance Standards for Antimicrobial Disk Susceptibility Tests. 8th Ed. **M2-A8**, 2003.

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19 Limitations of Diffusion Methods. Warning

Studies are not yet adequate to develop reproducible standards to interpret diffusion tests with other microorganisms not listed in the tables of this booklet.

Dangerously misleading results can occur when certain antimicrobials are tested against specific microorganisms (1,2). These combinations include the following:

- All beta-lactam antibiotics (except oxacillin, methicillin and cefoxitin) against methicillin-resistant staphylococci.
- Cephalosporins, aminoglycosides (except testing for high level resistance), clindamycin, and trimethoprim + sulfa against enterococci.
- First and second-generation cephalosporins, cephamycins and aminoglycosides against *Salmonella* and *Shigella* species.
- Cephalosporins against *Listeria* spp.
- Glycopeptides against *S. aureus* with reduced susceptibility to vancomycin.
- Penicillins, Cephalosporins and aztreonam against ESBL-producing *E. coli*, *K. pneumoniae*, *P. mirabilis* (except Temocillin).

Routine reporting of results from strains isolated **from the CSF** could be **dangerously misleading** for patient care in the following cases:

- Agents administered orally only
- 1st and 2nd generation cephalosporins (except Cefuroxime sodium)
- Clindamycin
- Macrolides
- Tetracyclines
- Fluoroquinolones

Some antimicrobials are associated with the **emergence of resistance** during prolonged therapy. As a consequence, isolates initially susceptible may become resistant within a few days after initiation of treatment. This occurs most frequently in:

- *Enterobacter*, *Citrobacter* and *Serratia* spp. with third generation cephalosporins and aztreonam.
- *Pseudomonas aeruginosa* with most antimicrobials.
- Staphylococci with quinolones.

Repeat testing in 3-5 days should be considered.

Essentially all isolates of *Enterobacter aerogenes*, *E. cloacae*, *Citrobacter freundii*, *Providencia* spp., *Proteus* spp. (except *P. mirabilis*), *Serratia marcescens*, *Pseudomonas aeruginosa*, possess the genes for Group I beta-lactamase production. Therefore, there is no useful information from demonstrating in vitro **induction** of the enzyme. Laboratories should focus on **repeat testing** (every 3-4 days or earlier depending on the patient's condition) of isolates repeatedly recovered from infected patients during therapy to detect **selection of clones** that constitutively produce **Group I beta-lactamase** (1).

References:

1) NCCLS. Performance Standards for Antimicrobial Disk Susceptibility Tests. 8th Ed. **M2-A8**, 2003.
2) CLSI. Performance Standard for Antimicrobial Susceptibility Testing 17th Inf. Suppl. **M100-S17**, 2007.

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20 Susceptibility Testing of Anaerobes

Increasing resistance to clindamycin and some beta-lactam agents among the Bacteroides group has been found at some hospitals.

Organisms recognized as virulent or commonly resistant, should be considered for testing. These include the *Bacteroides fragilis* group, *Prevotella* and *Porphyromonas* spp., *Clostridium perfringens, Cl. ramosum Cl. septicum* and *Cl. difficile* (7).

A hypervirulent strain of *Cl. difficile* ribotype 027 reported as cause of outbreaks in Holland, Canada, USA and the UK. The strain has a characteristic pattern, since it is resistant to CIPRO and ERY, but susceptible to Clindamycin and Metronidazole (7).

Cl. ramosum shows intrinsically low level resistance to vancomycin and linezolid. *Cl. innocuum* shows intrinsically low level resistance to vancomycin and daptomycin. *Cl. clostridioforme* shows intrinsically low level resistance to teicoplanin.

The recommended procedure for susceptibility testing of anaerobes by the diffusion method is the following:

- Use supplemented Brucella blood agar, it supports good growth for essentially all anaerobes. Brucella agar base is supplemented with 5 µg/ml haemin, 5% defibrinated sheep blood and 1 µg/ml vitamin K1 (haemin and vitamin K1 may be added before sterilisation).
- Direct suspension of colonies in broth to achieve a turbidity equivalent to a 1.0 McFarland standard ($3x10^8$ CFU/ml). Streak the surface of the agar with a cotton swab.
- Allow the inoculated plate to remain at room temperature (5-10 min.) until the surface of the agar looks dry. For some fastidious isolates that do not grow on control plates, pre-reduction of plates in an anaerobic environment may be necessary. Apply Neo-Sensitabs tablets.
- Invert the inoculated plate and incubate at 35°C in an anaerobic jar or alternative anaerobic environment, for 24-48 hours.

Zone diameter interpretative standards are correlated to the MIC break points recommended by the CLSI for anaerobic bacteria (1):

Anaerobes:

Supplemented Brucella blood agar. Inoculum: McFarland 1.0
Incubation in anaerobic environment, MIC break-points according to CLSI (M11-A7)

				Zone diameter in mm			Break-points MIC µg/ml	
NEO-SENSITABS				**S**	**I** b)	**R**	**S**	**R**
a)	Ampicillin	33 µg	AMP33	≥ 32	31-26	< 26	≤ 0.5	≥ 2
	Ampicillin+Sulbactam	30+30 µg	AM+SU	≥ 26	25-22	< 22	≤ 8 (4)	≥ 32 (16)
	Amoxycillin+Clav.	30+15 µg	AM+CL	≥ 28	27-23	< 23	≤ 4 (2)	≥ 16 (8)
	Cefotetan	30 µg	CFTTN	≥ 20	19-16	< 16	≤ 16	≥ 64
	Cefoxitin	60 µg	CFOXT	≥ 24	23-18	< 18	≤ 16	≥ 64
	Chloramphenicol	60 µg	CLR60	≥ 26	25-22	< 22	≤ 8	≥ 32
	Clindamycin	25 µg	CLIND	≥ 28	27-23	< 23	≤ 2	≥ 8
	Doxycycline	80 µg	DOXYC	≥ 26	25-22	< 22	≤ 4	≥ 16
	Ertapenem	10 µg	ERTAP	≥ 23	22-19	< 19	≤ 4	≥ 16
	Fucidin (Clostridia) *	100 µg	FUCID	≥ 30	-	≤ 29	≤ 2	≥ 4
c)	Imipenem	15 µg	IMIPM	≥ 24	23-20	< 20	≤ 4	≥ 16
	Imipenem+EDTA	15+750 µg	IM+ED	Detection of metallo-beta-lactamases				
	Linezolid	30 µg	LINEZ	≥ 21	-	-	≤ 4	-

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	NEO-SENSITABS			Zone diameter in mm S	I b)	R	Break-points MIC µg/ml S	R
	Meropenem	10 µg	MEROP	≥ 22	21-18	< 18	≤ 4	≥ 16
d)	Metronidazole	16 µg	MTR16	≥ 22	21-18	< 18	≤ 8	≥ 32
	Moxifloxacin	5 µg	MOXIF	≥ 23	22-19	< 19	≤ 2	≥ 8
a)	Penicillin Low	5 µg	PEN.L	≥ 24	23-18	< 18	≤ 0.5	≥ 2
	Piperacillin	100 µg	PIPRA	≥ 24	23-20	< 20	≤ 32	≥ 128
	Piperacillin+Tazobactam	100+10µg	PI+TZ	≥ 24	23-20	< 20	≤ 32 (4)	≥ 128(4)
	Tetracyclines	80 µg	TET80	≥ 28	27-23	< 23	≤ 4	≥ 16
	Ticarcillin	75 µg	TICAR	≥ 24	23-20	< 20	≤ 32	≥ 128
	Ticarcillin+Clavulanate	75+15 µg	TI+CL	≥ 24	23-20	< 20	≤ 32 (2)	≥ 128(2)
	Tigecycline	15 µg	TIGEC	≥ 18	17-15	< 15	≤ 4	≥ 16
	Vancomycin	5 µg	VAN.5	≥ 18	-	-	≤ 4	-

* Break-points have not been established by the CLSI.

a) Members of the *Bact. fragilis* group are resistant. Other gram negative anaerobes should be screened for beta-lactamase with nitrocefin, and if positive reported as penicillin/ampicillin resistant.

b) The intermediate range is established because of the difficulty of reading zone endpoints with some bacteria-antibiotic combinations and because of clustering of MICs at breakpoint concentrations.

c) Imipenem shows typically MIC´s of ≤ 0.12 µg/ml against *Bact. fragilis*. (Imipenem Neo-S zone > 30 mm). Strains showing higher imipenem MIC´s (2-4 µg/ml) may represent up to 7% of *Bact. fragilis* isolates (2) and although susceptible to the breakpoint of 4 µg/ml, they may sometimes conduct to therapeutic failure.

d) Resistance among strict anaerobes to metronidazole, in most cases represent laboratory error (3). Strict anaerobiosis is critical for accurate metronidazole susceptibility testing and this environmental failure is the most likely source of false-resistant results. The SFM recommends Metronidazole Neo-Sensitabs for sensitivity testing of anaerobes (5).

Beta-lactamase testing has limited utility in detecting resistance to certain beta-lactam agents among certain anaerobes. A positive nitrocefin test can predict resistance to penicillin and ampicillin. Other types of tests are not suitable for anaerobes (1).

Imipenem resistant and metronidazole resistant *Bact. fragilis* group strains have been detected in fecal samples using the diffusion method (4).

Detection of carbapenemase (metallo-ß-lactamase) production in the *B. fragilis* group is performed using Imipenem and Imipenem+EDTA as well as Meropenem/EDTA. Positive strains show inhibition zones ≥ 5 mm larger with Imipenem+EDTA compared to Imipenem and/or synergism between Meropenem and EDTA (8).

Since most anaerobic infections involve mixed aerobic-anaerobic flora with several different strains, it may be difficult to develop reliable predictive values for patient management.

When new problems are recognised or improvements in these criteria are developed, changes will be incorporated into future editions of this booklet and also distributed as informational supplements.

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Quality control limits for anaerobes:

Suppl. Brucella Blood Agar
Inoc.: McFarland 1.0. Anaerobic incubation

NEO-SENSITABS		CODE	Zone diameter in mm: Bact. fragilis ATCC 25285	MIC µg/ml	B. thetaiotaomicron ATCC 29741	MIC µg/ml
Amoxycillin+Clavulanate	30+15 µg	AM+CL	32 – 38	0.5	35 – 41	0.5
Cefoxitin	60 µg	CFOXT	27 – 33	8	22 – 27	16
Chloramphenicol	60 µg	CLR60	29 – 34	8	32 – 37	16
Clindamycin	25 µg	CLIND	30 – 37	1	25 – 30	4
Imipenem	15 µg	IMIPM	33 – 40	0.06	34 – 41	0.12
Linezolid	30 µg	LINEZ		4		4
Meropenem	10 µg	MEROP	29 – 35	0.12	31 – 36	0.12/0.25
Metronidazole	16 µg	MTR16	29 – 36	0.5	30 – 36	1
Tetracyclines	80 µg	TET80	33 – 39	0.25	22 – 26	16
Penicillin Low	5 µg	PEN.L	9 – 14	16	9 – 14	16
Piperacillin	100 µg	PIPRA	22 – 27	4 (8)	22 – 28	8 (16)
Piperacillin+Tazobactam	100+10µg	PI+TZ	26 – 32	0.25/4	26 – 32	4/4

References:

1) NCCLS Methods for Antimicrobial Susceptibility Testing of Anaerobic Bacteria, 7th Ed. Approved Standard, **M11-A7**, 2007.
2) Edwards R.et al: Mechanism responsible for reduced susceptilility to imipenem in *Bacteroides fragilis.* J. Antimicrob. Chemother., **38**, 941 - 951, 1996.
3) Cormican M.G. et al: False resistance to metronidazole by E-test, among anaerobic bacteria. Diagn. Microbiol. Infect. Dis., **24**, 117 - 119, 1996.
4) Fang H. et al: Detection of imipenem-resistant and metronidazole-resistant *Bact. fragilis* group strains in fecal samples. Clin. Microbiol. Infect., **5**, 753-8, 1999.
5) SFM. Comite de l'Antibiogramme de la SFM. Communiqué 2006.
6) Aldrige K.E.: Detection of carbapenemase production in the *B. fragilis* group using the MBL E-test strip: correlation with broth microdilution testing. Clin. Microbio. Inf., **11**, Suppl. 2, 625, 2005.
7) Kuijper E.J. et al: Clostridium difficile ribotype 027, toxinotype III, The Netherlands. Emerg. Infect. Dis., **12**, 827-830, 2006.
8) Bogaerts P. et al: Phenotypic and genotypic analysis of carbapenem-resistant B. fragilis strains isolated in Belgium. Presentation d-246, ICAAC 2007, Chicago, USA.

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21 Susceptibility Testing of Yeasts

The CLSI M27-A2 reference broth microdilution method requires 48 hours' incubation and can be cumbersome to perform for most laboratories. Therefore there is interest in the validation of the agar-based methods of disk diffusion for the antifungal susceptibility testing of yeasts.
Approximately 10 years ago Rosco Diagnostica developed the first disk diffusion method using modified Shadomy agar with Amphothericin B, Fluconazole, Fluorocytosine, Itraconazole and Ketoconazole Neo-Sensitabs.

Studies with fluconazole and ketoconazole have, however, shown a good correlation between in vitro and in vivo results using animal models (1). In vitro resistant strains isolated from clinical treatment failure were clearly more difficult to treat in the animal models than typical susceptible strains.

Troillet et al (1993) demonstrated a good correlation between MICs and zone diameters using a fluconazole disk on Yeast Nitrogen agar. Barry et al (2) defined a simple disk diffusion test for rapid determination of the susceptibility of *Candida* spp. to fluconazole, using RPMI-1640-glucose agar.

Quindós et al (3) in a multicenter survey of antifungal resistance using Neo-Sensitabs antifungals, found a good correlation between the 12 Spanish laboratories involved in the survey. Other papers about the use of antifungal Neo-Sensitabs have been presented or published elsewhere (4,5,6,7,14,15,18,19,20,21,24,27,33,34,36,37,39,40,44).

Subsequent studies modified the procedure to use Mueller-Hinton Agar supplemented with 2 % glucose, because this medium is more readily available in the microbiological laboratory. Further studies showed that the addition of 0.5 µg/ml methylene blue made the zones of inhibition clearer and easier to read, particularly with the azole drugs.
Finally this technique was approved by the CLSI (formerly NCCLS) as the disk diffusion method of choice for performing antifungal susceptibility testing of yeasts (CLSI document M44-A).

Lee (25 and Barry (26) using the CLSI technique could read the inhibition zones after 24 hours' incubation. 97 % of results were in agreement with those of the reference test.
Vandenbosche et al. (24) showed that the disk diffusion method using Neo-Sensitabs had the best agreement with the CLSI reference method (better than Etest).
In a study using 282 *Candida* spp. Rementeria et al. (37) comparing paper disks and Neo-Sensitabs containing Fluconazole and Voriconazole found that the results were equivalent, but the zones of inhibition were best defined and easier to read with Neo-Sensitabs.

In a recent study by Espenel-Ingroff (50), comparing the Neo-Sensitabs disk method to both CLSI-reference broth microdilution and disk diffusion for testing susceptibilities of 10-20 isolates each of Candida (8 species) and *C. neoformans* to 5 antifungal agents: amphothericin B, caspofungin, fluconazole, itraconazole and voriconazole, found that agreement by breakpoint category of Neo-Sensitabs results with CLSI method M27-A2 was 95.5 %.
Even though Amphotericin testing was found to work on MH-GMB agar (38), results with amphothericn B might be diffucult to assess by the current diffusion method. Consequently Rosco Diagnostica is developing a prediffusion method with Amphothericin B Neo-Sensitabs that we expect will detect all strains with reduced susceptibility to amphothericin B.

According to Pfaller et al. (31) the fluconazole disk diffusion method using MHGMB agar performed acceptably for testing *C. neoformans*, when compared to the reference microdilution method.
Recently Carillo et al. (51) tested Caspofungin and Voriconazole Neo-Sensitabs against 184 clinical isolates of *Candida* spp. and other medically important yeasts, finding the expected results.
Diekema et al. (52) evaluated Etest and disk diffusion against the broth microdilution reference method using 2171 isolates of *Candida* spp. from 60 medical centres worldwide. Posaconazole results with disk diffusion correlated well with the CLSI broth microdilution method demonstrating categorial agreements of 98 % (interpretations S ≥ 17 mm and R ≤ 13 mm).

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21.1 Procedure according to CLSI

Colonies may be taken from CHROM agar Candida medium or from non-differential media as potato dextrose agar.

1) Inoculum density and inoculation

It is important to standardize the inoculum. Therefore, use a McFarland 0.5 inoculum.
Plates may be dried for 20 minutes at 35 °C before inoculation.
Inoculate Mueller-Hinton agar plates supplemented with 2 % dextrose (glucose) and 0.5 µg/ml methylene blue with the undiluted 0.5 McFarland Standard, using a sterile cotton-wab dipped into the suspension, removing excess fluid by pressing aginst the tube. Inoculate the agar by streaking while rotating the plate, swabbing over the entire agar surface.

2) Incubation time

Reading of the test should be made as soon as possible - i.e. for yeasts after **not more than 18-24 hours**. A longer incubation time may result in false resistance against imidazoles/azoles. Plates should always be examined after overnight incubation - and if inhibition zones are visible, they must be measured. If growth is not yet visible with particular strains, the plates may be reincubated for up to 24 hours more. For *Cryptococcus* spp. incubate at 30 °C for 42-48 hours.

3) Criteria for measuring inhibition zones

For the polyenes (Amphotericin B, Nystatin) the clear zone with no visible growth is measured. Colonies inside the zone of inhibition must be considered resistant mutants.
For azoles/imidazoles, Caspofungin and Terbinafine (Candida) measure the zones must be measured up to colonies of normal size. There is often a zone of growth of partially inhibited colonies (smaller) at the edge of the real zone. They are not resistant mutants.
Fluorocytosine cannot be tested with MH-Glucose, Methylene Blue agar, because of the presence of antagonists in the agar. It may be testing using Shadomy agar or similar agars free of antagonists.
Individual large colonies inside the zone are usually resistant mutants.

21.1.1 Interpretation Tables for Yeasts (MH-GMB)

21.1.1.1 Interpretation according to CLSI breakpoints

When using the procedure recommended by the CLSI for the diffusion test (M-H agar + 2 % glucose + 0.5 µg/ml methylene blue and McF. 0.5 inoculum) the interpretation will be as follows (25,26,29,30,44,47,48,49,50):

MH Glucose Methylene Blue Agar
Inoculum McFarland 0.5 undiluted
MIC breakpoints according to CLSI (M44-A)

			Zone diameter in mm			Break-points MIC µg/ml	
NEO-SENSITABS	**POTENCY**	**CODE**	**S**	**I**	**R**	**S**	**R**
Amphotericin B	10 µg	AMPHO	≥ 15	14-10	< 10	≤ 1	≥ 2
Caspofungin	5 µg	CASP5	≥ 15	14-12	≤ 11	≤ 2	> 2
Fluconazole *	25 µg	FLUCZ	≥ 19	18-15 (DD)	≤ 14	≤ 8	≥ 64
Itraconazole (50)	8 µg	ITRAC	≥ 23	22-14 (DD)	≤ 13	≤ 0.12	≥ 1
Ketoconazole	15 µg	KETOC	≥ 28	27-21	≤ 20	≤ 0.12	≥ 0.5
Posaconazole (52)	5 µg	POSAC	≥ 17	16-14	≤ 13	≤ 1	≥ 4
Voriconazole	1 µg	VOR.1	≥ 17	16-14	≤ 13	≤ 1	≥ 4

DD = Dosis Dependent

* *C. krusei* should always be reported as resistant to fluconazole (no matter the zone size).

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Azole resistance
Development of azole resistance may be a progressive phenomenon that may be related to the accumulation of stepwise mutations, with MIC of fluconazole being the first to increase after the selective presure made by any azole (13). Isolates with fluconazole MIC > 4 µg/ml are in general less susceptible to other azole drugs, but different patterns of decreased susceptibility are found. On the other hand, isolates with fluconazole MICs ≤ 2 never show decreased susceptibility to other azole drugs (13).
C. glabrata, *C. tropicalis*, *C. albicans* and *C. parapsilosis* showing fluconazole MICs ≥ 64 µg/ml (R) corresponding to zones of inhibition ≤ 14 mm with Fluconazole Neo-Sensitabs showed cross-resistance with voriconazole (MICs of ≥ 2 µg/ml) (46). On the other hand *C. Krusei* (fluconazole R) are currently voriconazole susceptible (no cross-resistance).

EUCAST is working on the harmonization of breakpoints, however, there is no agreement with MIC breakpoints from CLSI. In studies comparing methods, interpretive categories misclassification may be seen caused by differences in MIC breakpoints (41,43).

21.1.1.2 Interpretation according to EUCAST MIC breakpoints:

MH Glucose Methylene Blue Agar
Inoculum McFarland 0.5 undiluted
MIC breakpoints according to EUCAST (tentative)

NEO-SENSITABS	POTENCY	CODE	Zone diameter in mm S	I	R	Break-points MIC µg/ml S	R
Amphotericin B	10 µg	AMPHO	≥ 18	17-13	≤ 12	≤ 0.25	≥ 2
Fluconazole *	25 µg	FLUCZ	≥ 28	27-23	≤ 22	≤ 2	> 4
Itraconazole (50)	8 µg	ITRAC	≥ 23	22-14	≤ 13	≤ 0.12	≥ 1
Ketoconazole	15 µg	KETOC	≥ 30	29-23	≤ 22	≤ 0.12	≥ 1
Posaconazole (52)	5 µg	POSAC	≥ 22	21-16	≤ 15	≤ 0.25	≥ 2
Voriconazole	1 µg	VOR.1	≥ 22	21-16	≤ 15	≤ 0.25	≥ 2

All MIC breakpoints from EUCAST are tentative (41).

* *C. krusei* should always be reported as resistant to fluconazole.

21.1.2 Interpretation table fo Local treatment

In local treatment of fungal infections a high concentration of antifungal is placed at site of the infection. Consequently other MIC breakpoints and zone interpretations should be used in those cases.

Local Treatment
MH Glucose Methylene Blue Agar or Shadomy
McFarland 0.5 inoculum

Susceptible	≥ 20 mm	≥ 15 mm	≥ 10 mm
Intermediate	12-19 mm	10-14 mm	-
Resistant	≤ 11 mm	no zone	no zone
	Ciclopirox Clotrimazole Econazole Fluconazole Isoconazole Ketoconazole Miconazole Tioconazole Terbinafine	Natamycin Nystatin Itraconazole	Griseofulvin

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21.1.3 *Candida* spp. Quality Control (MH-GMB)

Quality control limits using Candida strains on modified Shadomy agar or MH-GMB agar are included in the table below:

Mueller-Hinton Glucose Methylene Blue Inoculum McFarland 0.5 undiluted Incubation 18-24 hours			
	Zone diameters (mm)		
NEO-SENSITABS	***C. albicans*** **ATCC 90028**	***C. parapsilosis*** **ATCC 22019**	***C. krusei*** **ATCC 6528**
Amphotericin B	18-23	20-26	15-21
Caspofungin	15-22	13-23	16-22
Fluconazole	28-39	22-33	-
Itraconazole	21-30	19-26	16-22
Ketoconazole	31-42	26-35	22-29
Posaconazole	24-34	25-36	23-31
Voriconazole	31-42	25-36	23-31

21.2 Procedure using Shadomy modified agar

This technique developed by Rosco approx. 10 years ago (8) uses the following procedure:

1) Media
The modified Shadomy Agar contains Yeast Nitrogen Base, glucose and asparagine, and is buffered with phosphate to pH 7.0. Chloramphenicol has been added to avoid bacterial contamination.

2) Inoculum and inoculation technique
Prepare a McFarland 0.5 suspension and ilute further 1:1 with saline.
For *C. krusei* use a McFarland 0.5 suspension diluted 1:10 with saline.
For *Cryptococcus* spp. use an inoculum equivalent to McFarland 1.0 undiluted.
0.5 ml (9 cm plate) or 1 ml (14 cm plate) of the above suspensionis poured onto the agar surface (flooding) and excess liquid is removed immediately with a pipette. Thereafter the open plate is dried at 35 °C for 10 minutes before applying the Neo-Sensitabs disks on the agar surface.

3) Incubation time
Reading of the test should be made as soon as possible - i.e. for yeasts after **not more than 18-24 hours**. A longer incubation time may result in **false resistance** against imidazoles/azoles. Plates should always be examined after overnight incubation - and if inhibition zones are visible, they must be measured. If growth is not yet visible with particular strains, the plates may be reincubated for up to 24 hours more. For *Cryptococcus* spp. incubate at 30° C for 42-48 hours.

21.2.1 Interpretation Tables for Yeasts (modified Shadomy agar)

The following zone size interpretation should be used with using this procedure with MIC breakpoints according the CLSI M44-A.

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21.2.1.1 Interpretation according to CLSI breakpoints

Shadomy Modified Agar
Inoculum McF. 0.5 diluted 1:1, flooding
MIC breakpoints according to CLSI M44A

NEO-SENSITABS	POTENCY	CODE	Zone diameter in mm S	I	R	Break-points MIC µg/ml S	R
Amphotericin B	10 µg	AMPHO	≥ 15	14-10	< 10	≤ 1	≥ 2
Caspofungin	5 µg	CASP5	≥ 15	14-12	≤ 11	≤ 2	> 2
Fluconazole *	25 µg	FLUCZ	≥ 22	21-15 (DD)	≤ 14	≤ 8	≥ 64
Fluorocytosine	**1 µg**	FLU.1	≥ 20	19-12	≤ 11	≤ 4	≤ 32
Fluorocytosine	**10 µg**	FLU10	≥ 30	29-23	≤ 22	≤ 4	≤ 32
Itraconazole	8 µg	ITRAC	≥ 23	22-14 (DD)	≤ 13	≤ 0.12	≥ 1
Ketoconazole	15 µg	KETOC	≥ 30	29-23	≤ 22	≤ 0.12	≥ 0.5
Posaconazole	5 µg	POSAC	≥ 17	16-14 (DD)	≤ 13	≤ 1	≥ 4
Voriconazole	1 µg	VOR.1	≥ 17	16-14 (DD)	≤ 13	≤ 1	≥ 4

DD: Dosis dependent.

21.2.1.2 Interpretation according to EUCAST MIC breakpoints

MIC breakpoints according to EUCAST
Shadomy Modified Agar
Inoculum McF. 0.5, diluted 1:1, flooding

NEO-SENSITABS	POTENCY	CODE	Zone diameter in mm S	I	R	Break-points MIC µg/ml S	R
Amphotericin B	10 µg	AMPHO	≥ 18	-	-	≤ 0.25	-
Caspofungin	5 µg	CASP5	≥ 15 *	-	-	≤ 1	-
Fluconazole *	25 µg	FLUCZ	≥ 28	-	-	≤ 2	-
Fluorocytosine	**1 µg**	FLU.1	≥ 26	-	-	≤ 1	-
Voriconazole	1 µg	VOR.1	≥ 22	-	-	≤ 0.25	-

* Tentative

21.2.2 *Candida* spp. Quality Control (modified Shadomy Agar)

Quality control limits using ATCC strains on modified Shadomy agar are included in the table below:

NEO-SENSITABS	*C. albicans* ATCC 64548	*C. albicans* ATCC 64550	*C. parapsilosis* ATCC 22019	*C. krusei* ATCC 6528
Amphotericin B	18-23	19-24	20-26	15-21
Caspofungin	15-22	-	13-23	15-22
Fluconazole	36-42	11-17	30-36	10-16
Fluorocytosine 1 µg	34-40	26-33	35-43	9
Itraconazole	25-31	9	28-35	16-22
Ketoconazole	36-44	21-29	41-49	20-26
Posaconazole	28-39	-	25-36	23-31
Voriconazole	31-42	-	28-37	16-25

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22 Veterinary Practice CLSI

Interpretation of the Antibiogramme with Neo-Sensitabs
MIC break-points according to CLSI (M31-A3) 2007
Inoculum according to Kirby-Bauer / confluent colonies

	NEO-SENSITABS	POTENCY	CODE	Zone diameter in mm S	I	R	Break-points MIC µg/ml S	R
	Amoxicillin+Clav.	30+15 µg	AM+CL					
	Staphylococcus spp.			≥ 26	-	≤ 25	≤ 4/2	≥ 8/4
	Other organisms			≥ 20	19-17	≤ 16	≤ 8/4	≥ 32/16
	Amikacin	40 µg	AMIKA	≥ 20	19-17	≤ 16	≤ 16	≥ 64
	Amoxycillin	30 µg	AMOXY					
	Enterococcus spp.			≥ 20	-	≤ 19	≤ 8	≥ 16
	Other organisms			≥ 20	19-17	≤ 16	≤ 8	≥ 32
	Ampicillin	33 µg	AMP33					
	Enterobacteriaceae			≥ 20	19-17	≤ 16	≤ 8	≥ 32
	Staphylococcus spp.	(use Pen Low)						
	Enterococcus spp.			≥ 20	-	≤ 19	≤ 8	≥ 16
	Streptococcus spp. (not *S. pneumoniae*)			≥ 30	29-21	≤ 20	≤ 0.25	≥ 8
	Listeria monocytogenes			≥ 26		≤ 25	≤ 2	≥ 4
	Mannheimia haemolytica			≥ 32	31-26	≤ 25	≤ 0.25	≥ 1
c)	Apramycin	40 µg	APRAM	≥ 23	22-20	≤ 19	≤ 4	≥ 16
c)	Bacitracin	40 U	BACIT	≥ 20	19-17	≤ 16	-	-
c) e) h)	Cefadroxil	30 µg	CFDRO	≥ 23	22-20	≤ 19	≤ 8	≥ 32
e) h)	Cefazolin	60 µg	CFZOL	≥ 23	22-20	≤ 19	≤ 8	≥ 32
f)	Cefoxitin	60 µg	CFOXT					
	S. aureus			≥ 25	-	≤ 24	OxaS	MecA pos
	Coag. neg. staph.			≥ 28	-	≤ 27	OxaS	MecA pos
a) c) h)	Ceftriaxone (Cefoperazone)	30 µg	CETRX	≥ 20	19-17	≤ 16	≤ 8	≥ 32
h)	Cefpodoxime	30 µg	CFPOX	≥ 25	24-21	≤ 20	≤ 2	≥ 8
c) h)	Cefquinome	30 µg	CFQUI	≥ 23	22-20	≤ 19	≤ 2	≥ 8
h)	Ceftiofur	30 µg	CFTIO	≥ 23	22-20	≤ 19	≤ 2	≥ 8
c) h)	Cefuroxime	60 µg	CEFUR	≥ 23	22-20	≤ 19	≤ 8	≥ 32
c) e) h)	Cephalexin	30 µg	CFLEX	≥ 20	19-17	≤ 16	≤ 8	≥ 32
c) e) h)	Cephalothin	60 µg	CLOTN	≥ 23	22-20	≤ 19	≤ 8	≥ 32
	Chloramphenicol	60 µg	CLR60					
	S. pneumoniae			≥ 28	-	≤ 27	≤ 4	≥ 8
	Streptoccoccus spp.			≥ 28	27-21	≤ 20	≤ 4	≥ 16
	Other organisms			≥ 25	24-21	≤ 20	≤ 8	≥ 32
i)	Clindamycin	25 µg	CLIND					
	Staphylococcus spp.			≥ 26	25-23	≤ 22	≤ 0.5	≥ 4
c)	Doxycycline	80 µg	DOXYC	≥ 23	22-20	≤ 19	≤ 4	≥ 16
	Enrofloxacin	10 µg	ENROF	≥ 23	22-17	≤ 16	≤ 0.5	≥ 4
i)	Erythromycin	78 µg	ERYTR					
	Streptococcus spp.			≥ 28	27-24	≤23	≤ 0.25	≥ 1
	Other organisms			≥ 26	25-23	≤ 22	≤ 0.5	≥ 8
	Florfenicol	30 µg	FFC30					
	Cattle			≥ 20	19-17	≤ 16	≤ 2	≥ 8
	Swine			≥ 22	21-19	≤ 18	≤ 2	≥ 8
c)	Flumequine	30 µg	FLUME	≥ 20	19-17	≤ 16	≤ 2	≥ 4

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		NEO-SENSITABS	POTENCY	CODE	Zone diameter in mm S	I	R	Break-points MIC µg/ml S	R
c)		Fucidin	100 µg	FUCID					
		Plain agar			≥ 28	27-24	≤ 23	≤ 1	≥ 4
		Blood agar			≥ 26	25-23	≤ 22	≤ 1	≥ 4
c)		Furazolidone	50 µg	FURAZ	≥ 23	22-20	≤ 19	≤ 4	≥ 8
		Gentamicin	40 µg	GEN40	≥ 25	24-21	≤ 20	≤ 2	≥ 8
		Kanamycin	100 µg	KANAM	≥ 23	22-20	≤ 19	≤ 16	≥ 64
c)	i)	Lincomycin	19 µg	LINCO	≥ 26	25-23	≤ 22	≤ 2	≥ 8
c)	i)	Lincomycin/Neomycin (Albiotic Forte)	15+60 µg	LIN+N	≥ 20	19-17	≤ 16	≤ 2/4	≥ 5/16
	i)	Linco-spectin	15+200µg	LI+SP	≥ 20	19-17	≤ 16	≤ 4/32	≥ 16/64
		Imipenem	15 µg	IMIPM	≥ 20	19-17	≤ 16	≤ 4	≥ 16
c)		Marbofloxacin	5 µg	MAR.5	≥ 20	19-15	≤ 14	≤ 1	≥ 4
b)	c)	Metronidazole (anaerobes)	16 µg	MTR16	≥ 28	27-24	≤ 23	≤ 4	≥ 8
c)		Naf-Pen-Strep	5+2+20 µg	N+P+S	≥ 20	19-17	≤ 16	≤ 1/1/4	≥ 2/2/16
c)		Neomycin	120 µg	NEOMY	≥ 23	22-20	≤ 19	≤ 6	≥ 25
c)		Nitrofurantoin (nitrofurans)	260 µg	NITRO	≥ 23	22-20	≤ 19	≤ 32	≥ 128
c)		Novobiocin	5 µg	NOVO5					
		Blood agar			≥ 13	12-11	≤ 10	≤ 2	≥ 4
		Plain agar			≥ 16	15-14	≤ 13	≤ 2	≥ 4
f)		Oxacillin	**1 µg**	OXA.1					
		Coag. neg. staph.			≥ 18	-	≤ 17	≤ 0.25	≥ 0.5
		S. aureus			≥ 13	12-11	≤ 10	≤ 2	≥ 4
		S. pneumoniae (penicillin)			≥ 20	-	≤ 19	≤ 0.06	≥ 0.12
c)		Oxolinic acid	10 µg	OXOLI	≥ 20	19-17	≤ 16	≤ 4	≥ 8
		Penicillin/Novo	10 U + 30 µg	PEN+N					
		Mastitis			≥ 18	17-15	≤ 14	≤ 1/2	≥ 4/8
		Other organisms			≥ 17	-	≤ 16	≤ 1/2	≥ 4/8
		Penicillin Low	5 µg	PEN.L					
		Staphylococcus spp.			≥ 26	-	≤ 25	≤ 0.12	≥ 0.25
		Streptococcus viridans			≥ 26	25-13	≤ 12	≤ 0.12	≥ 4
		Beta haemolytic			≥ 26	-	-	≤ 0.12	-
		Other organisms			≥ 18	17-11	≤ 10	≤ 1	≥ 4
		Pirlimycin	10 µg	PIRLI	≥ 18	-	≤ 17	≤ 2	≥ 4
		Rifampicin	30 µg	RIFAM					
		S. pneumoniae			≥ 28	27-24	≤ 23	≤ 1	≥ 4
		Enterococci			≥ 26	25-23	≤ 22	≤ 1	≥ 4
		Spectinomycin (Pasteur., Haemoph.)	200 µg	SPECT	≥ 18	17-15	≤ 14	≤ 32	≥ 128
		Spiramycin	200 µg	SPIRA	≥ 26	25-23	≤ 22	≤ 2	≥ 8
c)		Streptomycins	100 µg	ST100					
		Pseudomonas spp.			≥ 32	-	≤ 31	≤ 6	≥ 25
		Other organisms			≥ 26	25-23	≤ 22	≤ 6	≥ 25
		Sulphonamides (U)	240 µg	SULFA	≥ 23	22-20	≤ 19	≤ 256	≥ 512
		Tetracyclines	80 µg	TET80					
		Streptococcus spp.							
		Cattle			≥ 26	25-23	≤ 22	≤ 2	≥ 8
		Swine			≥ 30	29-27	≤ 26	≤ 0.5	≥ 2
		Other organisms			≥ 23	22-20	≤ 19	≤ 4	≥ 16
		Tiamulin	30 µg	TIAMU					
		Spirochaetae			≥ 28	27-24	≤ 23	≤ 1	≥ 4
		Actinobacillus			≥ 11	-	no zone	≤ 16	≥ 32
c)		Tiamulin+Tetra	30+15 µg	-	≥ 16	15-14	≤ 13	≤ 8/4	≥ 16/8
		Ticarcillin	75 µg	TICAR					
		Ps. aeruginosa			≥ 16	-	≤ 15	≤ 64	≥ 128
		Gram neg. ent.			≥ 20	19-17	≤ 16	≤ 16	≥ 128

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	NEO-SENSITABS	POTENCY	CODE	Zone diameter in mm S	I	R	Break-points MIC µg/ml S	R
	Ticarcillin+Clavulanate	75+15 µg	TI+CL					
	Ps. aeruginosa			≥ 16	-	≤ 15	≤ 64/2	≥ 128/2
	Gram neg. enteric organisms			≥ 20	19-17	≤ 16	≤ 16/2	≥ 28/2
	Tilmicosin	80 µg	TILMI					
	Bovine RD			≥ 18	17-15	≤ 14	≤ 8	≥ 32
	Swine RD			≥ 15	-	≤ 14	≤ 16	≥ 32
c)	Trimethoprim	5.2 µg	TRIME	≥ 20	19-17	≤ 16	≤ 4	≥ 16
g)	Trimethoprim+Sulfa	5.2+240 µg	TR+SU					
	S. pneumoniae			≥ 32	31-27	≤ 26	≤ 0.5/9.5	≥ 4/76
	Systemic infection			≥ 32	31-27	≤ 26	≤ 0.5/9.5	≥ 4/76
	Urine			≥ 28	27-24	≤ 23	≤ 2/38	≥ 4/76
c)	Tylosin	150 µg	TYLOS	≥ 26	25-23	≤ 22	≤ 4	≥ 16
	Vancomycin	5 µg	VAN.5	≥ 16	15-14	≤ 13	≤ 4	≥ 32

Remarks:

a) Results with Ceftriaxone are valid for Cefoperazone.

b) Metronidazole 16 µg is the representative of the nitroimidazole-group, including ronidazole, ornidazole, ipronidazole, and moxnidazole. Results obtained with metronidazole are applicable to the others.

c) MIC break-points have not yet been given by the CLSI.

d) From August 2005, the FDA no longer allow the use of enrofloxacin for treating infections in poultry (to avoid development of resistance in *Campylobacter* spp.).

e) Results of cephalothin susceptibility tests are used to predict susceptibility to the first generation cephalosporins, such as cephadroxil and cephalexin.

f) Results of Cefoxitin and Oxacillin with staphylococci are used to predict susceptibility to cloxacillin. Cefoxitin resistant staphylococci should be reported as resistant to all beta-lactams. In case of discordant results between cefoxitin and oxacillin, report the strains as resistant (R).

g) The results of Trimethoprim+Sulfa can be used to predict the susceptibility of other potentiated sulphomanides with thrimethoprim.

h) For detection of ESBL (CTX-M) and AmpC beta-lactamases (CMY) in *Salmonella* spp. see Chapter 16 on "Detection of Beta-Lactamases" (7).

i) Routine screening of clindamycin inducible resistance in staphyloccci/streptococci should be performed (double disk/induction test, page 89) (6). Results are also valid for lincomycin.

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